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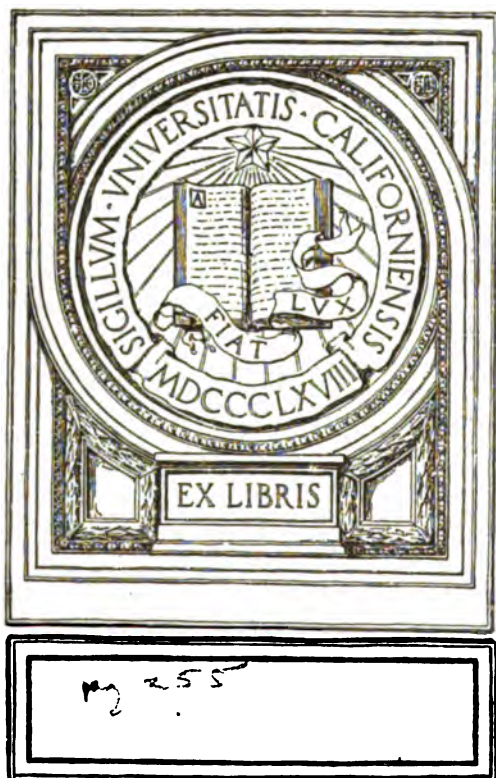
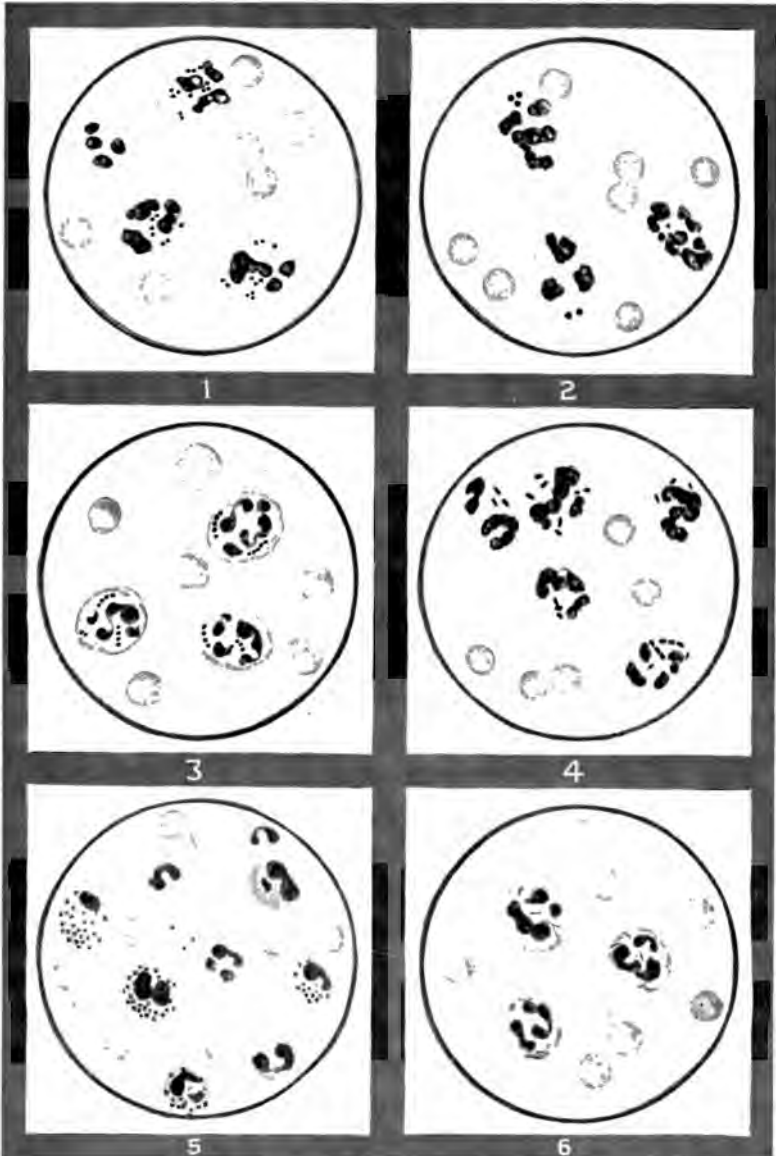


PLATE I.



Demonstrating phagocytosis of various bacteria as observed in the determination of the opsonic index. 1, *Staphylococcus*; 2, *Micrococcus candidans*; 3, *Streptococcus pyogenes*; 4, *Colon bacillus*; 5, *Gonococcus*; 6, *Tubercle bacillus*. (See p. 234.)

APPLIED IMMUNOLOGY

THE PRACTICAL APPLICATION OF SERA
AND BACTERINS PROPHYLACTICALLY,
DIAGNOSTICALLY AND THERAPEUTICALLY

WITH AN APPENDIX ON
SERUM TREATMENT OF HEMORRHAGE, ORGANO THERAPY
AND CHEMOTHERAPY

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TO VIND
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To

ALFRED STENGEL, M.D.

PROFESSOR OF MEDICINE IN THE UNIVERSITY OF PENNSYLVANIA

**IN GRATEFUL RECOGNITION OF HIS WISE COUN-
SEL AND KINDLY ADVICE THIS VOLUME IS
RESPECTFULLY DEDICATED BY THE AUTHORS**

PREFACE

DESPITE the enormous strides, experimentally and clinically, that have marked the progress in serological and bacteriological research in medicine for the past quarter of a century, leading to results of the highest clinical value diagnostically and therapeutically, three facts stand out in bold relief: First, that the average practitioner's knowledge of biological therapeutics is a "dangerous thing" because he does not "drink deep of the Pierian spring"; second, insufficient practical instruction is given to students in our medical schools in view of the prevalent practice of this mode of diagnosis and treatment; third, authors have sadly neglected to give a concise presentation of immunology in its entirety for the practical grasp and comprehension of students and practitioners. The vast majority of standard works on the subject of "immunity" are exhaustive treatises on the experimental and theoretical phases of the subject and are often unintelligible and of little value to the average practicing physician.

The result of the above has been that pharmaceutical firms have assumed the function of the Department of Therapeutics in our medical colleges, not always impartially and to the best interests of medical science.

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Obviously, licentiates to practice medicine should receive instruction of a definite and practical nature in this important branch of modern medicine, or immunology should receive a place in the classification of specialties, a fact which the importance of the subject may command.

In this book it has been the aim of the authors purposely to omit most of the experimental research and to present theories only in so far as they may assist in a more thorough comprehension of biological prophylaxis, diagnosis and therapeutics.

The primary object has been to crystallize and detail the practical phases of serum and bacterin applications in medicine, thereby enabling the student and general practitioner, with even a slight laboratory experience, to appreciate the significance of, and more competently apply the principles underlying, immunology. In order to render the treatise more complete allusion has been made in places to certain allied substances that have been utilized from time to time in attempts at immunization, with a consideration of their merits and demerits.

The interest and close association of blood transfusion, organotherapy and administration of salvarsan and neosalvarsan to the main subject have prompted the authors to devote an appendix to their discussion.

The reader who may have his enthusiasm aroused

and is ambitious to enter more deeply into the realms of immunity is referred to the systems of Kolle and Wassermann, and Kraus and Levaditi, or the works of Ehrlich and Bolduan, and Bordet and Gay on "Studies in Immunity"; Emery on "Immunity and Specific Therapy"; Wright on "Studies on Immunization"; Simon on "Infection and Immunity"; Citron on "Immunity," and Vaughan on "The Relation of Anaphylaxis to Immunity and Disease."

B. A. THOMAS

R. H. IVY

WILLIAM PEPPER LABORATORY OF
CLINICAL MEDICINE,
PHILADELPHIA, 1915

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APPLIED IMMUNOLOGY

I

INTRODUCTION

IMMUNITY AND IMMUNIZATION—NATURAL AND ACQUIRED IMMUNITY—ACTIVE AND PASSIVE IMMUNIZATION—MECHANISM OF THE PRODUCTION OF IMMUNITY—HISTORY AND DEVELOPMENT OF IMMUNOLOGY

Definition of Immunity.—*Immunity is the resistance manifested by man and various animal species to infectious microorganisms or other foreign proteins.* It is influenced by numerous factors, as changed environment, physical condition of the animal, species, idiosyncrasies, virulence of the prevalent microbe, etc. Conversely, the absence of this resistance implies susceptibility. Occasionally, hypersusceptibility to certain proteins is observed and to this state of supersensitiveness Richet has applied the term "anaphylaxis" (see Chapter IV).

Two kinds of immunity are recognized, *natural* and *acquired*.

Natural Immunity.—The natural or spontaneous resistance of the animal organism to disease is only relative, never absolute. The ability of animals to

ward off disease varies with different species and among individuals of the same family. Under normal conditions, an animal may be protected indefinitely from infection. Nevertheless, if his vital resistance be permitted to fall or he be exposed to a virulent infection, his defences may crumble instantly and disease be contracted. On the other hand, the natural immunity of certain species to infection is remarkable; the negro to yellow fever and most of the lower animals to the venereal diseases.

In this connection allusion should be made to *local immunity*. By this we mean a natural state of certain organs or tissues, prevalent from birth, due to "infective tolerance." For example, the mouth and anterior urethra normally harbor many different pathogenic bacteria without ill effect, owing to life-long local tolerance with resultant immunity. Introduce some of these germs into the synovial membrane of a joint or into the peritoneal cavity and a virulent infection results. Again, the intestinal tract tolerates colon bacilli normally in numbers, which, if access be gained to the urinary tract, may precipitate a grave pathological process.

Infections common in warm-blooded are rare among cold-blooded animals and *vice versa*. Vertebrates and invertebrates are not subject to similar infections. Field mice are susceptible to glanders,

while house mice are immune. Tuberculosis is more prevalent among Jersey than Holstein cattle. Birds and reptiles are not necessarily subject to the same diseases that victimize man. Thus the problem of natural immunity presents many interesting phases, but still lacks an absolutely satisfactory solution.

Explanations for the existence of natural immunity are founded on the protection afforded: first, by the external and internal surfaces of the body; second, by inflammatory processes; third, by natural antibacterial and antitoxic substances, and fourth, by the natural metabolic activity or vital resistance of the organism.

Bacteria are unable to penetrate the unbroken cutaneous epithelium, but may reach the subcutaneous tissues through abrasions although microscopical in size, through sudoriferous and sebaceous ducts and glands although their secretions are mildly antibacterial, and through the hair follicles. In the subcutaneous tissues, bacterial encroachment is further combated by cellular proliferation and extravasated plasma containing serum, fibrinogen, and leucocytes. Bacteria entering the nasal and oral passages encounter in the mucus and saliva both physical and chemical barriers. The gastric, biliary and pancreatic juices exert antibacterial and neutralizing functions.

Inflammation, a manifestation of tissue injury, is

a process designed to resist infection, if that be its cause, and proves successful for mechanical and immunological reasons, if the reactive forces of the individual be capable. The invading bacteria develop a condition of positive chemotaxis and leucocytes swarm to the battle-field. Already the bacteria have stimulated the tissue cells to the production of specific substances or antibodies and many bacteria are killed by lysis (see Chapter XI); the remainder, by virtue of sensitization with their specific antibodies (opsonins of Chapter XX), are ingested and destroyed by the phagocytes (see Chapter XVII). While the mortal conflict between bacteria and bacteriolysins and phagocytes is being waged, inflammatory exudate and proliferation of fixed tissue cells occur and raise barriers to the further extension of the morbid process. Coincidentally, the antibodies are formed in excess and impregnate the blood-serum, establishing the phenomenon of immunity.

There is little evidence pointing to the presence of natural antitoxic substances in animals, although they have been claimed to occur to a limited extent in horses and more abundantly in children and adults. Natural antibacterial substances are more extensively demonstrable in the tissue fluids and blood-serum. Buchner has given the name "alexins" to these normal bacteriolytic substances. They appear to be

identical with opsonins and may be increased by active immunization.

Acquired Immunity.—Acquired immunity is that condition of protection against disease, resulting from recovery from infection or arising by virtue of artificial inoculation. It may be produced in two ways, namely, by *active or passive immunization*.

Active immunization signifies the process by which the bodily cells of an animal are stimulated by a toxin or foreign body (antigen) to the production of certain other bodies (antibodies) specific against the given foreign substance (see Chapter II). Thus the animal is actively concerned in the elaboration of its own antibodies, hence the process is termed active. Untreated disease terminates in either one of three ways—death, recovery, or chronicity. If the virulence of the infection is great and the dose large or overwhelming, the animal succumbs, especially if his vital resistance be slight. If the infection is relatively avirulent, even though the dose be large, recovery although protracted may take place provided the animal's resistance is great. If the degree of virulence of the infection be merely the average, but the vital resistance of the animal be only mediocre, its cellular activity or infective dose may prove inadequate for the normal generation of sufficient specific antibodies to insure immunity, and chronic invalidism results. Thus

in patients suffering from chronic infections, particularly, and also in certain acute conditions, the number of specific antibodies may be materially increased and convalescence shortened or recovery insured by *artificial inoculation* employing homologous bacteria. This result may be achieved by injecting the bacteria with a hypodermic syringe directly into the tissues, or by carefully regulated and graduated *auto-inoculation*, by manipulations, as massage, hyperæmia, etc., of the infected area. The discovery of the feasibility of producing auto-inoculation in patients afflicted with a localized infection was one of great magnitude and vital consideration in the study and correct interpretation of infection and immunity, particularly in connection with bacterin therapy. By its utilization difficult diagnoses have been established, successful treatment conducted in selected cases and in constitutional diseases, clinical interpretations correctly deduced, and proper treatment applied (see Chapter XXI). It is evident, therefore, that when an individual recovers from an infectious disease he enjoys for an indefinite time immunity against repeated attacks due to the same infective organism. It can also be readily understood that immunity against particular diseases can be conferred by artificial inoculation, using the specific bacteria, viruses, etc. (prophylactic inoculation or vaccination).

The methods by which immunity may be acquired through active immunization are as follows, enumerated in their order of efficiency:

1. Inoculation with virulent living bacteria, typified by the subcutaneous injection of spirilla of Asiatic cholera.

2. Inoculation with attenuated or relatively avirulent microorganisms. This method is exemplified by vaccination against smallpox and antirabic inoculation.

3. Inoculation with dead bacteria. This embraces the prevalent practice of bacterin as prophylactic (vaccine) therapy, notable examples of which include bacterial inoculations against typhoid fever, plague, tuberculosis, furunculosis, carbunculosis, etc. A higher immunity may be produced by supplementing the dead bacterins with inoculations of attenuated and finally living virulent bacteria.

4. Inoculation with the excreted bacterial products. The injection of the horse with the tetanus toxin for the production of antitoxin is the familiar example of this method.

5. Inoculation with the disintegrated products of dead bacteria (autolysates). Little of value has attended this procedure and for practical purposes it may be disregarded.

Passive immunization signifies the process by which immunity is acquired when artificial antisera are injected into the animal body (see Chapter V). Thus the inoculated animal plays no part in the production of the antibodies or antitoxin which he receives, and the process is termed passive. Hence the animal is injected with the specific cellular products (antitoxin) of another animal previously actively immunized, and by a process of simple neutralization the toxins in the diseased animal are destroyed and immunity conferred. In passive immunization the antibodies bear a close chemical combination to the cells. The acquired immunity of passive immunization is of vastly shorter duration than that resulting from active immunization.

MECHANISM OF THE PRODUCTION OF IMMUNITY

There is a group of foreign chemical substances, conveniently styled antigens, to which the animal body reacts in a definite manner. This group must be differentiated from and not confounded with the poison group, as the common poisons are not antigens. The group embraces the foreign proteins, including the bacterial proteins, also certain complex and more or less unknown bodies, as bacterial toxins, parenteral proteolytic ferments, enzymes and other animal and vegetable toxic substances. Following the subcutaneous, intra-abdominal or intravenous injection of an

appropriate quantity of such an antigen, after a variable interval, during which a leucopænia may exist, leucocytosis supervenes. After an incubation period of a few days to several weeks, it is found that the body fluids, particularly the blood-serum, react in a novel manner, that is, they possess the property of neutralizing the antigen. Although these are known biological facts, explanations as to when and how these antibodies are formed and when and how they unite with their specific antigens are pure speculation. Nevertheless, immunologists generally have accepted the ingenious so-called *side-chain theory* of Ehrlich, which conception, although regarded as too visionary by some, has sufficed in a remarkable manner as a working basis for almost all the great problems and discoveries in serology (see Chapter III). On the other hand, Metchnikoff's doctrine of phagocytosis (Chapter XVII), not in its original simplicity, plays a not unimportant rôle in the mechanism particularly of active immunization in view of the researches of the opsonic school. The efficiency of a polynuclear leucocytosis in pneumonia has long been recognized as of momentous prognostic value and undoubtedly is intimately concerned in the production of immunity.

The *bactericidal* and *cytolytic substances* normally present in the blood-serum and body fluids of some animals, and capable of marked increase by antigenic

inoculations, are extremely important considerations in immunology (see Chapter XI).

Other notable antibodies conspicuous in serological work are agglutinins (Chapter IX), important in certain bacterial agglutination tests, and precipitins (Chapter X), of value in the identification of bloods in forensic medicine. The relationship of these specific antibodies to the immunity of the body is in doubt.

The production of anaphylaxis, hypersusceptibility or supersensitiveness to all proteins save gelatin, and its association with immunization contributes a most interesting and important subject and is fully discussed in Chapter IV.

HISTORY AND DEVELOPMENT OF IMMUNOLOGY

Historically, the first reference to any attempt at protection against disease by the utilization of biological products was made by Mithradates, who, it is alleged, took small quantities daily of certain poisons in order to render himself immune. Similarly, the custom of hunters of certain wild tribes to inoculate themselves systematically with snake venom to safeguard the effects of snake bites is well known.

Immunization, however, was not placed on a truly practical basis until the eighteenth century, although the practice had existed in the East a long time previously. Lady Montagu, the wife of the British Am-

bassador to Constantinople, permitted her son to be inoculated against smallpox with the matter from variola pustules and subsequently, in 1721, introduced the method in England. Despite the brilliant results that followed, the practice encountered bitter opposition, owing to the fact that although it protected the inoculated it did not prevent conveyance of the disease in a virulent form to the uninoculated, and was finally prohibited by law.

Sixty years later, the attention of Jenner was directed to a peculiar disease of the udders of cows, from which the hands of milkmen became infected, rendering them immune to smallpox. Jenner investigated the subject for a number of years, and in 1796 inoculated a boy with "cow-pox," after which inoculation with smallpox showed him to be immune. Two years later, in 1798, Jenner published his classical report, which was soon followed by systematic and universal vaccination against the world's greatest scourge. Although the causative organism in the virus of smallpox has not been discovered, even to this day, Jenner firmly established the doctrine that it is possible to confer immunity against an infectious disease by the employment of a modified *materies morbi*.

Almost a century passed before any further notable advance occurred in immunology. Indeed it required the stimulus of the era of bacteriology to pro-

mote research along this line. Schwann showed the relationship between decomposition of organic bodies and microorganisms. This was supplemented by Pasteur's work on fermentation and the yeast fungus. In 1863 Davaine pointed out that certain bacilli described in the blood by him thirteen years before were the causative factors in anthrax. Thirteen years later, Koch succeeded in growing these bacilli on an artificial medium in pure culture, and anthrax was reproduced in animals.

The next problem was to attenuate the bacteria, that is, preserve their identity and life, at the same time reducing their virulence, so that inoculation would not result fatally. In 1880, Pasteur succeeded in preparing a "vaccine" from attenuated anthrax bacilli and inoculated sheep, thereby rendering them immune.

In 1885, after much animal experimentation, Pasteur inoculated the first human subject against rabies. The etiological microorganism of the virus of this disease is still undetermined and the procedure of antirabic inoculation has undergone no material change.

Thus far the belief prevailed that immunity occurred only as the result of recovery from disease. Salmon and Smith at this time demonstrated that immunization could be produced by the products of

bacterial growth. Supplementing the work of Traube and Gscheidlen, in 1887, Buchner showed the specificity of the bactericidal properties of blood-serum.

In 1888, Roux and Yersin, and Kitasato discovered the toxins respectively of diphtheria and tetanus. In 1890, Behring discovered antitoxin in the serum of animals immunized against the toxin of diphtheria, thus furnishing the first antitoxic serum. In the same year, with the collaboration of Kitasato, immunity to tetanus was conferred on mice from the serum of rabbits inoculated with tetanus toxin. In the following year with Wernicke, he immunized other animals with antidiphtheric serum. Behring, therefore, discovered the whole principle underlying serum therapy in its relationship to modern therapeutics and serological studies, and became thereby its honored founder.

The following year Ehrlich demonstrated antibodies in the serum of animals inoculated with vegetable poisons, as ricin, abrin and crotin, and three years later Calmette claimed similar results with snake venom.

Although it was recognized, even before Behring's discovery, that the serum of animals inoculated with certain bacteria possessed a specific anti-infectious effect or protection, it was about this time, 1892, that Metchnikoff called attention to a particular substance,

"stimulin," in antibacterial sera, capable of stimulating leucocytes to increased ingestion and destruction of bacteria. Metchnikoff claimed that immunity depended upon this property of phagocytosis.

It was at this time that Buchner described "alexins," and Bordet "sensibilisators" in sera and their rôle in the process of immunization. Numerous foreign albuminous substances were utilized for the production of antibodies and conceptions respecting the nature of immunity definitely distinguished two types, *active* and *passive*. Moreover, it was realized that *exotoxigenic* bacteria were adaptable chiefly to passive, and *endotoxigenic* bacteria to active, immunization.

In 1895, Pfeiffer demonstrated his phenomenon of bacteriolysis, thus dealing a severe blow to Metchnikoff's doctrine. While the discussion was still warm over Pfeiffer's discovery, in the next year Gruber and Durham directed attention to the diagnostical value of "agglutination" of bacteria in their specific antiserum and Widal described the serodiagnosis of typhoid fever. The following year, Kraus described other antibodies, more or less closely related to bacteriolysins and agglutinins, which he styled "precipitins."

The year 1896 is notable in immunological annals as marking the advent of antityphoid inoculation by Wright. Previously, Ferran and Haffkine had employed attenuated living cultures, prophylactically,

against cholera. Wright's work, however, marked a new epoch, since he demonstrated that antibodies may be produced by inoculation with dead bacteria. The following year, Haffkine immunized himself against the plague by inoculation with a sterilized culture of *B. pestis*. Up to this time, bacterial inoculations on the human had been employed solely in a prophylactic capacity. Wright enjoys the distinction of being the first to realize that any bacterium responsible for local disease and capable of isolation in pure culture may be employed in the form of a bacterial suspension or bacterin to cure the disease it causes.

Seven years later, Wright and Douglas, taking advantage of Leishman's studies on comparative phagocytosis, showed that phagocytosis does not occur save in the presence of serum, thus claiming a specific sensitization of bacteria by certain substances in the blood-serum to which the name "opsonins" was given. An ingenious and clever laboratory method was devised whereby the measure of the ratio of phagocytability could be determined and this was styled the "opsonic index." The work of Wright attracted universal attention and popularized active immunization by bacterin therapy to an unprecedented and world-wide extent.

Synchronously with Wright's studies, the discoveries and advances in serology were truly remarkable.

In 1898, Belfanti and Carbone discovered that the serum of horses immunized with the blood of rabbits was very toxic for these animals. The analogy between these cytotoxins or cytolytins and the specific antibodies formed after bacterial inoculations was immediately realized. Experiments by Bordet, Ehrlich and Morgenroth demonstrated the presence of hæmolysins in the serum of animals inoculated with red blood-cells, and the interesting and important phenomenon of hæmolysis or solution of erythrocytes was demonstrated.

Almost all tissue cells were employed in research work for the production of their specific antibodies, and to these various heterogeneous elements the name "antigens" was applied. Thus, leucotoxins, spermotoxins, trichotoxins, syncytiolysins, hepatolysins, nephrolsins and neurotoxins were produced and their influence as causative factors in the pathology of certain diseases, as hepatitis, nephritis, etc., became a much mooted subject in scientific research. In 1900, Uhlenhuth appreciated the formation of specific cytotoxins for carcinoma and sarcoma in patients, and employed such sera therapeutically.

The year 1902 is memorable in serology as marking the discovery of the "complement-fixation reaction" by Bordet and Gengou. They demonstrated that no hæmolysis can occur if the thermolabile ele-

ment of hæmolysin be absorbed or fixed in the interaction between antigen and antibody. This discovery proved to be of the greatest importance and practical value and underlies many modern serological clinical tests.

Wassermann, in 1906, the year following the discovery of the *Treponema pallidum*, put the complement-fixation or deviation reaction of Bordet and Gengou to a practical and successful test for the diagnosis of syphilis, and the so-called "Wassermann reaction" was the result. Subsequently the principles employed in Wassermann's technic have been applied to many other infectious diseases, notably gonorrhœa.

In 1909, Ascoli, employing Traube's stalagmometer, announced the "meiostagmin reaction," and demonstrated the feasibility of differentiating between fluids containing mixtures of antigens and antibodies by measurement of the drops contained in given volumes. The method has been utilized in the diagnosis of typhoid fever, tuberculosis, malignant tumors, foot-and-mouth disease, and for the detection of many lipoidal substances.

Finally, reference should be made to certain paradoxical reactions that have been observed in immunology during recent years. Instead of the immunity which commonly follows the injection of a foreign protein substance into an animal, occasionally, al-

though immune bodies have been demonstrated to be present, the inoculation of heterogeneous substances, without apparent reason, produces a singular effect, in consequence of which the previously treated organism reacts differently from the normal. To this reaction von Pirquet has given the name "allergy." Allergy may be expressed by either a refractory state of the organism, namely, immunity, or by supersensitiveness, that is, anaphylaxis. Examples of anaphylactic reactions are recognized in "Arthus' phenomenon" in rabbits, "Theobald Smith's phenomenon" in guinea-pigs and "serum disease" in man. Less important, although more practical illustrations are commonly seen in the cutaneous and ophthalmic tuberculin reactions of tuberculous individuals, the mallein reaction in glanders, the luetin reaction in syphilis, the intradermic reaction in typhoidal subjects, the sero-diagnosis of cancer, etc. *etc.*

In this connection, the hypothesis of Friedberger that a number of pathological processes are due to the occurrence of albumen anaphylaxis, and the coincident poisons of infections are referable to the influence of the concurrent anaphylotoxin, must receive serious consideration.

II

ANTIGENS AND ANTIBODIES

TOXINS AND ANTITOXINS—AGGLUTININS—PRECIPITINS—
LYSINS—ISOCYTOLYSINS—OPSONINS—ANTIFERMENTS—
AUTO-ANTIBODIES—ALLERGY AND ANAPHYLAXIS

WE will now discuss briefly the various defensive properties of the fluids and tissues of the body in resisting the invasion of disease-producing substances. The latter are usually termed *antigens*, while the specific substances produced by the body-cells to neutralize and thus render the antigens innocuous are termed *antibodies*. By the term *antigen*, therefore, is meant an organic foreign substance, usually protein in nature, the product of animal or vegetable cells, which on being introduced into the body has the property of stimulating the production by the body-cells of a specific substance or *antibody* which unites chemically with it and thus neutralizes it. Antigens may be of various types. They may consist of the soluble products of bacterial growth, of substances bound up in the bodies of the bacteria themselves, of protein and lipoid extracts of animal tissues, etc. These different forms of antigens produce corresponding types of antibodies which differ in their methods of attacking the antigens. Antibodies thus specifically produced,

as distinguished from those naturally existing in the normal animal, are called *immune bodies*, and the animal is said to be immune to the particular antigen in question.

VARIOUS FORMS OF ANTIBODIES

(a) *Toxins and Antitoxins*.—*Toxins* are the soluble products of bacterial and plant growth, also the secretion of certain reptiles, and in the case of some bacteria are the means through which the deleterious effects of the bacteria are brought about. *Antitoxins* are specific substances produced in the blood-serum whereby the action of bacterial toxins is antagonized.

(b) *Agglutinins*.—Many forms of bacteria, when introduced into the body, stimulate the body-cells to produce specific substances called agglutinins, which have the property of causing the bacteria to lose their motility and to mass together in clumps. This phenomenon is of great practical importance in the diagnosis of certain bacterial infections. For example, the blood-serum of a patient affected with typhoid fever contains agglutinins, so that when it is brought in contact with typhoid bacilli agglutination of the latter will take place.

(c) *Precipitins*.—Precipitins are closely allied to agglutinins, and are antibodies which bring about the precipitation of soluble foreign proteins. They also

have considerable diagnostic importance, to which attention will be called later.

(d) *Lysins*.—Lysins are among the most important of all antibodies, both from the stand-point of protection against disease and also from the fact that many diagnostic tests are based upon their action. Lysins are antibodies that bring about the solution of cells, and the term *cytolysin* may therefore be applied to them all. Lysins which bring about the solution of bacteria are termed *bacteriolysins*; those which cause solution of red blood-cells are known as *hæmolysins*. Thus, the terms *cytolysis*, *bacteriolysis* and *hæmolysis* should be readily understood. Lysins are formed as the result of introduction of bacteria or of cells of an animal of an alien species into the body. Thus, the red blood-cells of the sheep, when introduced into the body of the rabbit, produce antibodies or hæmolysins in the rabbit's blood-serum, which when brought subsequently in contact with sheep's corpuscles under certain conditions will dissolve the latter.

(e) *Isocytolysins*.—It has been found that lysins may not only be produced against the cells of an animal of a foreign species (heterolysins) but also that the introduction into the body of cells of an animal of the same species will cause the formation of specific cytolytic antibodies (isocytolysins).

(f) *Opsonins*.—Opsonins are substances present

in the blood-serum which prepare bacteria for phagocytosis or absorption by the leucocytes. *Normal opsonins* are present in every serum. Opsonins which are called forth by the introduction of particular bacteria are called *immune opsonins* or bacteriotropins. They are more resistant to heat (thermostable) than normal opsonins.

(g) *Antiferments*.—These are antibodies which resist the action of the different ferments. Owing to their presence, self-digestion by the various cells of the body is probably prevented. Thus for the ferments, pepsin, trypsin, rennin, lipase, etc., we have the corresponding antipepsin, antitrypsin, antirennin, antilipase, etc.

(h) *Auto-antibodies*.—It has further been shown that an animal can be made to form antibodies against its own cells when these are introduced parenterally.

ALLERGY

To the altered condition of an animal into whose tissues has been introduced an antigen or foreign cell product, von Pirquet has given the term *allergy*. Under allergic phenomena are therefore included all subsequent reactions on the part of the body as a result of the parenteral introduction of foreign protein materials, *i.e.*, introduction by other channels than through the gastro-intestinal tract. An animal thus

treated is said to be "sensitized," *i.e.*, its cells have produced an excess of antibodies against the particular foreign material introduced.

ANAPHYLAXIS

Anaphylaxis is the term given by Richet to a state of hypersusceptibility or supersensitiveness to the action of a foreign protein, after the animal has been first "sensitized" by an injection of the protein in question. The phenomena of anaphylaxis will be discussed in greater detail in Chapter IV.

III

EHRlich'S SIDE-CHAIN THEORY

OUR present view of the mechanism of the defensive processes of the body against disease is based upon the *side-chain theory of Ehrlich*. This theory explains the interaction of the various antigens and antibodies and is almost completely supported by experimental evidence. It is most important to realize that the interaction between antigens and antibodies is of a chemical nature, *i.e.*, the antibody does not destroy the antigen but forms a chemical combination with it. Ehrlich's theory conceives of each body-cell as consisting of a central molecular complex or nucleus upon which its life depends and a number of processes or "side chains" capable of combining with foodstuffs for the nutrition of the cell and with foreign substances which might prove injurious to it. These side chains or processes are termed *receptors*. Each receptor has a special affinity for a certain kind of foodstuff or toxin. Certain receptors are common to all cells, while others are found only in special cells. It is also conceivable that some receptors may not be normally present but are formed only by the stimulation of certain forms of toxins. The toxic molecule which unites with the cell receptor consists of two groups, a *hapto-*

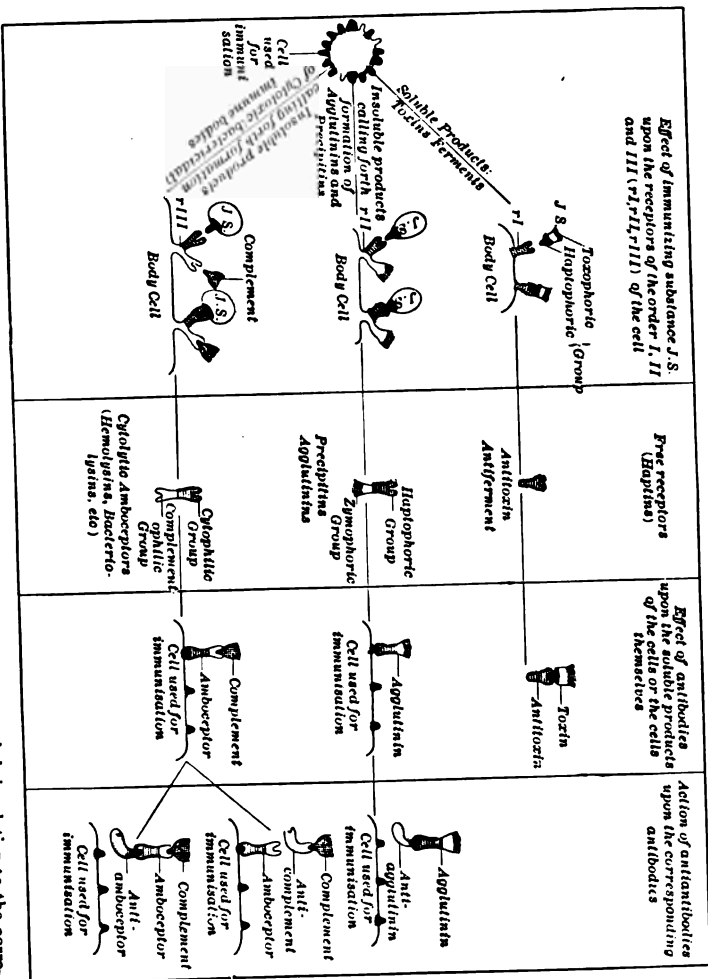


FIG. 1.—Diagrammatic representation of the structure of the different antibodies and their relation to the corresponding antigens. (Taken from Aechhoff) (Simon's "Infection and Immunity.")

phore group, which binds it to the cell receptor, and a *toxophore* group, which actually bears its toxic properties. When the receptor combines with the toxin molecule, the cell throws it off into the circulation, and similar receptors are formed to take its place. These, however, are formed in excess, and the cell throws them off also. These free receptors then unite with corresponding toxin molecules in the circulation. There is a receptor for each particular form of toxin molecule. Thus the diphtheria toxin combines only with the specific receptor provided for it, and will not unite with those intended for tetanus toxin. Ehrlich's theory, which at first only covered the simple union of toxin with antitoxin, was extended also to explain the action of more complex antibodies, and may be said to cover completely all forms of antigen-antibody action. There are three recognized types or orders of receptors, which are conveniently illustrated by the accompanying diagram (Fig. 1). Receptors of the first order possess only a single haptophore or combining group, by which they unite with the haptophore group of the corresponding antigens. To this order belong antitoxins and anti-ferments. Receptors of the second order, in addition to the haptophore group, possess a second or *zymophore* group, by means of which the anchored antigen can be subjected to further change. Under this head belong agglu-

tinins and precipitins. Receptors of the third order possess two combining groups, and are termed *amboceptors*. The first of these anchors the antigen to the cell and is known as the *cytophilic* group, while the second combines with the complement of the blood-serum and is known as the *complementophilic* group. Thus, for the action of receptors of the third order, an outside substance, the complement of the blood-serum, is necessary. Among these are cytolytins (bacteriolysins, hæmolysins, opsonins, etc.). It is unnecessary to go into the experimental evidence that has been brought forward in support of Ehrlich's theory of immunity, but numerous investigators have fully confirmed his views by experiments.

It is well known that poisons of known composition, such as alkaloids, glucosides, etc., when introduced into the body do not produce antibodies, thus differing essentially from true antigens. The true antigens are closely related to foodstuffs and consequently have an intimate chemical relation with the body-cell, which provides special receptors for them. The poisons, on the other hand, not having this special chemical affinity, are not closely bound to the cell-substance, but become physically stored up. Many of these substances can be recovered from the body by extraction, which could not occur if chemical union had taken place.

IV

ANAPHYLAXIS OR HYPERSUSCEPTIBILITY

IN connection with the administration of antisera for therapeutic purposes, the possibility of the occurrence of the phenomenon known as anaphylactic shock must be borne in mind.

The phenomenon of protein sensitization was recognized by Vaughan, von Behring and others for many years before the name *anaphylaxis*, under which it is now generally known, was given by Richet in 1911. The term indicates absence of protection, as opposed to prophylaxis. Anaphylaxis consists in a series of apparently deleterious effects produced by a second injection of specific protein material into an animal that has been previously "sensitized" by a first injection of the same material. For instance, if an animal receive one injection of a foreign protein material, say of antitoxic serum, no deleterious effects are usually observed, but if this injection be repeated after several days, even in a very small dose, the second injection may be followed immediately by a marked reaction, such as convulsions and respiratory difficulty, in some cases with a fatal result. The anaphylactic phenomena usually follow when the second injection is given at least twelve days after the first.

One of the earliest investigators to devote special attention to this subject was Richet. He injected an animal with a dose of protein poison, and after complete recovery from the symptoms produced, by injecting a very much smaller dose which in a non-sensitized animal would have no deleterious effects, was able to cause death in a few hours. This hypersusceptibility in the sensitized animal Richet ascribed to the formation of a special antibody by the first injection, which on the second injection causes the splitting off of a highly toxic substance from the toxin injected, giving rise to the symptoms. Richet's conclusions were based on the assumption that the phenomena only occurred when toxic substances were injected. Arthus in 1908 showed that hypersusceptibility occurred when non-toxic protein material was used, such as normal horse serum, milk, etc. (Arthus' phenomenon). He also showed that the symptoms could only be produced by means of the same substance that was originally injected; *e.g.*, that an animal first sensitized with horse serum would not be affected with a subsequent injection of milk or other protein material, but only with horse serum. The studies of von Pirquet showed that a certain interval must elapse after the first injection of serum before a second injection will produce symptoms, and he concluded that this interval represented a period of incubation necessary for anti-

body formation. He applied this theory to the period of incubation and symptoms of infectious diseases, all of which may be regarded as anaphylactic phenomena. In short, according to von Pirquet, the causative agents of disease do not show their deleterious effects until antibodies are formed which split them up, setting free toxic substances. Theobald Smith observed that guinea-pigs, which had been injected with toxin-antitoxin mixture, were hypersensitive to subsequent injections with normal horse serum and rapidly succumbed to small doses of the latter. The most attractive and rational explanation of the phenomena following protein sensitization or anaphylaxis was first brought forward by Vaughan in 1907 (*Jour. Infectious Diseases*, 1907, iv, 476). Vaughan's views may be briefly stated as follows: Every protein molecule consists of a poisonous group and a non-poisonous or specific group. The latter group, when the molecule is introduced into the body, induces the development in the body of a specific proteolytic ferment, which has the power of splitting up similar protein molecules, setting free the poisonous or non-specific group. The poison thus set free produces the phenomena of anaphylaxis. Upon the first injection of a serum, therefore, the specific group of the molecule causes the formation in the body of a specific proteolytic ferment. The animal is now said to be "sensitized." If a second

dose of the same serum then be given, its molecule is split up by the proteolytic ferment, and the toxic constituent is set free, giving rise to anaphylaxis. There is no setting free of the poisonous elements of the protein molecule immediately after the first injection of serum, because the specific proteolytic ferment develops gradually in response to the stimulation of the specific group of the molecule. Hence the toxic element is only liberated in small amounts as the ferment is formed. But by the time a second injection of serum is administered, a considerable amount of the proteolytic ferment is stored up, and is ready to split up the molecules of the second injection and set free the toxin which gives rise to symptoms of anaphylaxis. The anaphylactic poison affects chiefly the nervous system, and particularly the respiratory centres. The reaction manifests itself in respiratory difficulty and convulsions, which may result fatally, and is particularly liable to occur in asthmatic persons. The injection of antitoxic horse serum has been known to produce anaphylactic phenomena in persons in whom attacks of asthma are induced by contact with horses. We do not yet know the nature of the anaphylactic poison. It is now recognized that not only serum, but all forms of toxic and non-toxic protein material, including milk, extracts of normal and pathologic ani-

mal tissue, and bacteria, may give rise to anaphylactic phenomena.

Anti-anaphylaxis.—If an animal receive a second injection of foreign protein material before the twelfth day after the first injection, a condition of resistance or decreased susceptibility may be set up (anti-anaphylaxis), and the symptoms of anaphylaxis will not occur. This resistance may be kept up by repeated injections at short intervals. This fact is of importance in the repetition of antitoxic serum for therapeutic purposes. The mechanism of anti-anaphylaxis is as yet unknown.

Passive Anaphylaxis.—Anaphylaxis may be brought about passively as well as actively. Thus, if one animal be sensitized by an injection of foreign protein and sufficient time be allowed to elapse for the formation of the specific protein-splitting ferments, introduction of its serum into a second animal will sensitize the second animal to the protein in question. Anaphylaxis will then follow immediately after the injection of the second animal with the protein.

The anaphylaxis reaction is made use of in the diagnosis of several diseases. The various tuberculin reactions of von Pirquet, Wolff-Eisner, Calmette, Moro and others depend on this hypersusceptibility. Tuberculous persons being “sensitized” by the tuberculous process, the application of tuberculin in these

cases will give rise to the various reactions. A similar reaction of diagnostic value occurs in gonococcic infection, a rise of temperature and other anaphylactic symptoms following the injection of gonococcus bacterin in persons suffering from this infection. Noguchi's luetin reaction in syphilis also is an anaphylactic manifestation.

Many of the phenomena of disease may be explained on the basis of anaphylactic or allergic phenomena. As pointed out by von Pirquet, the incubation period of an infectious disease is the time required by the body to form specific antibodies to the infecting organism. The onset of symptoms marks the setting free of the toxin, when the infecting organism is split up by the specific antibody. Studies in anaphylaxis have also furnished a very plausible explanation for certain individual idiosyncrasies. It is probable that hay fever and allied conditions are manifestations of anaphylaxis. The persons susceptible to these idiosyncrasies are probably sensitized by the pollen of plants and other substances, so that they manifest symptoms when placed in contact with small quantities of this proteid. In the same way may be explained idiosyncrasies to various articles of food, such as strawberries, pork, lobster, exposure to exhalation from horses, etc.

Certain precautions may be taken to avoid ana-

phylactic shock in the administration of serum for therapeutic purposes. Rosenau and Anderson point out that anaphylaxis is to be feared in all persons who have shown a tendency to asthma, or who have received previous injections of serum at least twelve days before. In these persons it is well to make a preliminary injection of a small trial dose of 0.1 to 0.2 c.c. of serum, and if no symptoms appear within two hours, the full dose may be given.

Serum Sickness.—The symptoms, grouped under the term “serum sickness,” occasionally seen after the injection of antitoxic serum in diphtheria and other diseases, are for the most part manifestations of anaphylaxis. These symptoms usually consist in fever, urticarial eruptions, lymphatic nodal enlargements, and kidney irritation. Usually these symptoms are transitory, but fatal cases have been reported. In order for these phenomena to follow a single injection, as occasionally happens, a large dose of serum must be employed; but the appearance of symptoms may occur after a very small second injection. The mild cases of serum sickness require no special treatment. With the refinement of preparation and concentration in small bulk of antisera, untoward symptoms following injection have become comparatively rare.

V

ANTISERA

PREPARATION OF ANTISERA—INDICATIONS FOR THERAPEUTIC USE OF ANTISERA

THERAPEUTIC antisera for protective and curative passive immunization fall into two general classes: (a) antitoxic sera, and (b) antibacterial sera, according to the mode of action of the bacteria against which they are directed.

Antitoxic sera are sera which are applied to the group of bacteria whose deleterious action depends upon the toxins separated from them in the process of growth. The best examples of this class of bacteria are the bacillus of diphtheria and the bacillus of tetanus.

Antibacterial sera are employed particularly in infections by those bacteria whose toxins are contained within the bacterial protoplasm (endotoxins). They act by destroying the bacteria themselves. Generally speaking, passive immunization has not been so successful in dealing with infections by bacteria belonging to this group, as with those whose action depends on separated or extracellular toxins.

PREPARATION OF ANTITOXIC SERA

When bacteria whose action depends upon toxins separated from them in the process of growth enter the body, they, as a rule, remain at the point of entrance, from which the toxins spread through the circulation. In response to the stimulation of the toxins, the body produces *antitoxins* in the blood-serum, by which their deleterious action is neutralized. The antitoxins at the same time render harmless to some extent the living bacteria at the point of entrance. This process is termed *active* immunization.

Antitoxin production is artificially induced by active immunization of suitable animals by subcutaneous, intraperitoneal or intravenous injections of the toxins of certain bacteria. The antitoxin thus formed not only protects the animal inoculated, but can also be removed from the original animal and used as a curative and protective agent against the same infection in other animals, by means of injections. The process in the second animal is known as *passive* immunization. So we distinguish *active* from *passive* immunization by the fact that in the former the animal produces its own antibodies to combat an infection, while in the latter the antibodies have been produced by another animal (see Chapter I).

As an example of the preparation of antitoxic sera, the method of artificial production of antidiphtheritic serum (diphtheria antitoxin) will be described.

First of all it is necessary to obtain a quantity of the toxin, separated from the bacteria. The diphtheria bacilli are grown in bouillon, and the fluid containing the toxin freed from bacteria by passage through a Berkefeld filter. The toxin strength of the fluid must then be measured. The unit of toxin strength is the smallest quantity necessary to kill a guinea-pig weighing 250 grammes in 5 days or less. This quantity of toxin is called the minimal lethal dose. It should be noted that not all strains of diphtheria bacilli form toxin suitable for the production of antitoxin. For the formation of antitoxic serum, the horse has been found to be the most suitable animal, but individual horses differ in the quantity and quality of antitoxin they are able to produce. No definite rules can be laid down for the active immunization of the horse against diphtheria toxin. But in a general way, small doses of the toxin are first injected, and gradually increased, until a very large quantity of the toxin can be injected at one time. In regulating the amount of toxin to be injected, one is guided by the effects of the previous dose, which are not shown immediately as a rule, but appear after a few days.

From time to time, small quantities of blood are removed from the jugular vein of the horse, and tested for antitoxic strength. When a sufficiently powerful antitoxic content has been demonstrated in the horse's

blood-serum, as described below, a large amount of blood is drawn off, and prepared for therapeutic purposes. The same horse can be used over and over again for the formation of antitoxin, if allowed a sufficient period for recuperation after each bleeding. The formation of a sufficiently potent antitoxic serum usually requires from six weeks to two months.

The strength of antitoxin is measured in units. The *antitoxic unit* is that quantity of horse's serum which will render harmless the injection of 100 minimal fatal doses of toxin. It is now ascertained what is the smallest quantity of toxin which when mixed and injected with 1 antitoxin unit will kill a guinea-pig of 250 grammes in 4 or 5 days. This quantity of toxin is then mixed with different dilutions of the horse's serum and injected subcutaneously into several guinea-pigs weighing 250 grammes each. Supposing that 1/1000 c.c. of the antitoxin neutralizes the dose of toxin so that the guinea-pig injected with this quantity remains alive, then 1 c.c. of the horse's serum is said to contain 1000 units of antitoxin. The strength of the antitoxic serum having been ascertained in this manner, it is placed in suitable quantities in syringes for therapeutic use. All these procedures are of course carried out under strict aseptic conditions. Antidiphtheric serum, like other antisera, loses its potency after variable lengths of time.

Antitetanic and other antitoxic sera are produced and tested in a similar manner, the horse being the animal usually immunized for their production.

PREPARATION OF ANTIBACTERIAL SERA

The bacteria belonging to this group do not act by means of extracellular toxins. Their deleterious effects depend upon toxins set free from the bacterial protoplasm when the organisms undergo disintegration (endotoxins). These toxins cannot be separated from the bacteria themselves *in vitro*, except to a very slight extent. At any rate it is doubtful if the substances which call forth the production of antibodies can be thus separated. Antibacterial sera are therefore produced by injecting the bacteria themselves into the body of the animal that is to be actively immunized. These are injected, usually at first inactivated, but also at times in an attenuated or even in their living state (Fig. 2), in gradually increasing doses until an antibacterial serum of suitable strength is obtained, when the animal is bled, and the serum preserved in ampoules containing convenient doses for therapeutic injection. The antibodies contained in antibacterial sera are not of the comparatively simple nature of those found in antitoxic sera, but belong to the second and third orders of Ehrlich. The action of antibacterial sera depends upon several factors, in-

cluding opsonins, bacteriolysins, and to some extent agglutinins and precipitins (see Chapters IX, X, XI and XX).

The standardization of dosage of antibacterial sera is therefore more difficult and uncertain than that of

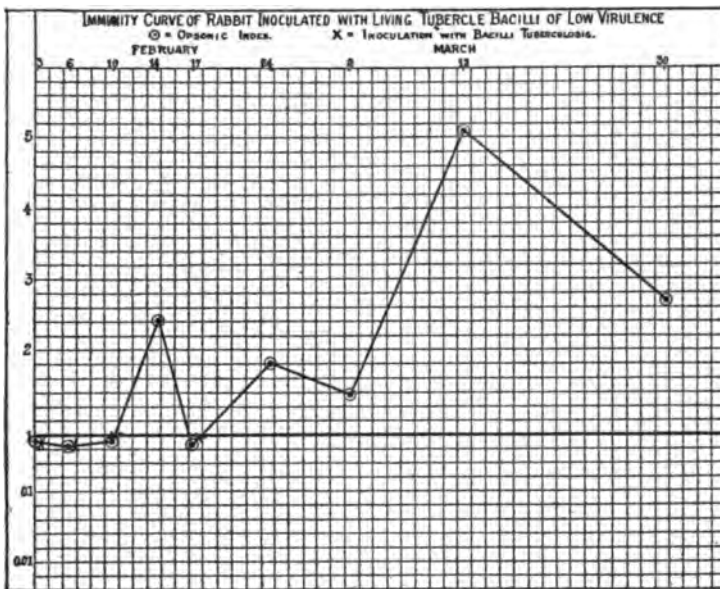


FIG. 2.—Illustrative of opsonic curve, showing immunity of rabbit inoculated with living tubercle bacilli of low virulence. O = opsonic indices; X = inoculations.

antitoxic sera, but the potency of an antibacterial serum can to some extent be gauged by determination of the opsonic index from time to time during the immunization of the animal (Fig. 3), and also by testing the agglutinating power of the serum, though the latter method is of only slight value as an indication of

therapeutic strength. The dosage of antibacterial sera is chiefly determined by the therapeutic effects obtained. In general, antibacterial sera are not of so much value as antitoxic sera, though in the case of a

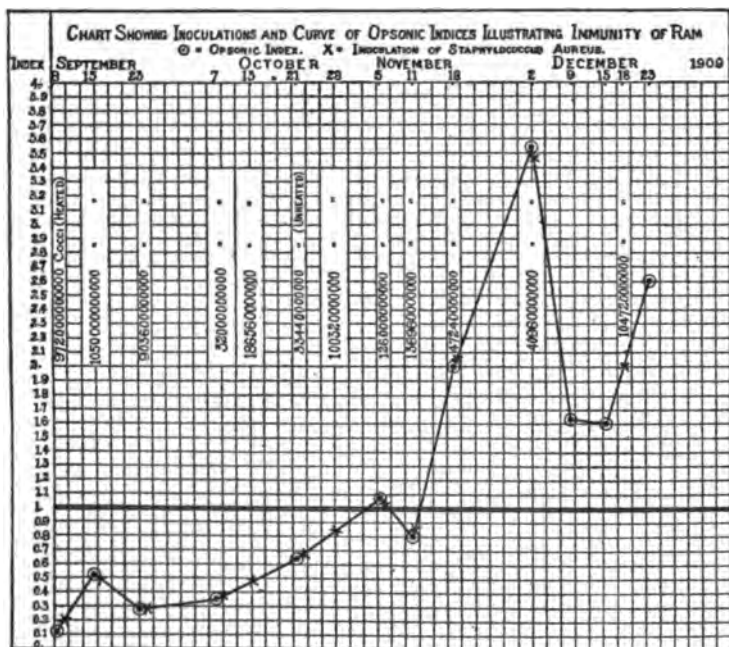


FIG. 3.—Illustrative of inoculations and curve of opsonic indices demonstrating immunity of ram. O = opsonic indices; X = inoculations of *Staphylococcus aureus*.

few organisms good results have been obtained. Active immunization offers a greater field of usefulness than passive immunization in infections of this type.

INDICATIONS FOR THE THERAPEUTIC USE OF ANTISERA

In the case of infections with bacteria which act through extracellular toxins, antitoxic sera have a

high therapeutic value both for protective and curative purposes. By their use in persons liable to be exposed to an infection, protection for a limited period of time may be conferred. This protection by passive immunization is not so durable as by active immunization, though the time necessary to acquire it is much less, a fact of considerable importance in some cases. It has been estimated, experimentally and clinically, that the duration of passive immunity persists from three to six weeks, while active immunization may endure for a year or two. Moreover, studies in passive immunization have shown that the duration of immunity from homologous antisera lasts three to four times as long as that from heterologous immune sera.

Antitoxic serum in some infections has a high curative value in persons already suffering from the disease, and indeed in certain infections is the most important means of treatment. In the case of other diseases, the value of the antitoxic serum as a curative agent is problematical.

Antibacterial sera, in infections where they are of any value at all, are indicated especially to overcome the immediate infection, owing to their rapid effects, while more lasting protection in this class of infections is conferred by active immunization which pursues a slower course. The indications for active and passive immunization in specific cases will be taken up later.

VI

ANTITOXIC SERA

ANTITOXIC sera are of recognized value particularly in diphtheria and tetanus, and are employed as routine therapeutic agents in these diseases.

Antitoxic sera have also been prepared and used from time to time in other infections with varying success. Examples of these are antigonococcic serum, antityphoid serum, antituberculosis serum, antidysenteric serum, anticholera serum, antibotulism serum. In addition, antitoxic sera have been employed in hay fever (antiphytotoxic sera), and against snake venoms (antizootoxic sera).

ANTIDIPHThERIC SERUM (DIPHTHERIA ANTITOXIN)

With antidiphtheritic serum are obtained the most brilliant results in the domain of serum therapy. Statistics from all sources attest its value. The serum is employed both for protective and curative purposes. The method of preparation of antidiphtheritic serum has already been given in Chapter V.

Protective Use of Diphtheria Antitoxin.—The average duration of immunity conferred by a protective dose of diphtheria antitoxin is three weeks. This varies with the severity of the epidemic present. In

ordinary epidemics a suitable protective dose is 500 units, injected subcutaneously.

Site and Method of Injection.—The usual sites of injection are under the skin of the abdomen, the thigh, or between the shoulder blades. The skin is thoroughly cleansed with alcohol, lifted with the thumb and forefinger, and the needle of the syringe quickly introduced beneath it. The serum is allowed to enter slowly, the needle quickly withdrawn, the puncture site compressed and stroked with a pledget of cotton soaked in alcohol, and the skin gently massaged. It is usually unnecessary to apply any dressing at the site of puncture of the skin. Infection rarely follows if asepsis has been observed. A protective dose of antitoxin should be given to all persons coming in close contact with a case of diphtheria, especially to children. Some hospitals require that all children admitted receive a prophylactic injection.

Von Behring's Method of Prophylaxis.—Recently von Behring has brought forward a new method of prophylaxis against diphtheria, by combined active and passive immunization. He injects a mixture consisting of strong diphtheria toxin and antitoxin in such proportions that the toxin is slightly in excess of the antitoxin. Experimental evidence proves that more lasting immunity is conferred by this means than by

the injection of antitoxin alone. Statistics showing the increased incidence of cases of diphtheria in large cities demonstrate the necessity for improvement in methods of prevention, and it is hoped that this method will fulfil its promise. Few statistics are available as yet upon which to base conclusions.

Curative Treatment of Diphtheria.—Antitoxin should be used as early in the disease as possible, for with every day of delay there is a great increase in the mortality rate. The death-rate is practically nil in patients who receive injections on the first day of the disease. The average dose in the ordinary case when seen on the first day is 5000 units, regardless of the age of the patient unless extremely young. In a severe case the dose should be 10,000 or 15,000 units. In cases seen later the dose should be proportionately larger, sometimes as much as 100,000 units being given. A single large dose of the serum is preferable to repeated smaller doses. Beneficial results should be seen from the injection in about eight hours, characterized by a subsidence of the throat symptoms and fall of the temperature and pulse-rate. Otherwise the injection may be repeated in larger dose. While the administration of antitoxic serum is by far the most important point in the treatment of diphtheria, other measures, such as rest in bed, liquid diet, sprays,

and cardiac and renal therapeusis, must not be omitted.

Diphtheria Carriers.—After disappearance of the clinical symptoms of diphtheria it is found that many cases still harbor the bacilli in the nose and throat for variable lengths of time, thus becoming sources whereby the disease is spread to others. Moreover, many persons harbor the organisms who have never had an attack of the disease. Schiotz, of Copenhagen, in 1909, after noticing that persons with staphylococcic infections of the nose and throat seldom contracted diphtheria, began to use, with good results, a spray made from a culture of *Staphylococcus aureus* in persons infected with diphtheria bacilli. Reports by many observers show almost uniformly good results from this treatment. Lorenz and Ravenel (*Jour. A. M. A.*, August 31, 1912, p. 690) recommend a fresh suspension of *Staphylococcus pyogenes aureus* in normal saline solution or a bouillon culture twelve hours old. This is used as a spray for the fauces, pharynx and nose, after the local signs of diphtheria have disappeared, while the cultures for the bacilli are still positive. The spray is repeated at four-hour intervals on two succeeding days, and continued thereafter until the cultures become negative to diphtheria bacilli. Untoward results are rarely seen. The period of quarantine after diphtheria is greatly shortened by

this method when compared with cases treated by anti-septic sprays alone, and many cases of "carriers" that have resisted all other forms of treatment have responded rapidly to it.

ANTITETANIC SERUM

Tetanus, like diphtheria, is a disease, manifestations of which depend upon a toxin separated from the tetanus bacilli in the process of growth. When a wound becomes infected with tetanus bacilli, the latter remain at the point of entrance and give off the toxin which is disseminated along the nerve sheaths until it reaches the spinal cord. Here the toxin combines with the cells of the central nervous system, and gives rise to the typical symptoms of the disease. This affinity of tetanus toxin for nerve tissue has an important bearing in the treatment of tetanus.

The method of artificially producing antitetanic serum is similar to that of producing antidiphtheric serum. Tetanus toxin is formed by growing tetanus bacilli anaërobically in bouillon for several days, the bacilli being then removed by passing the fluid through a Berkefeld filter. The filtrate is now injected into a horse, at first in small amounts (0.5 c.c.) mixed with a quantity of antitetanic serum. The doses are then gradually increased, the antitoxin being omitted after the first few injections. There are several methods of

standardization of tetanus antitoxin. The unit for the United States is the smallest amount of immunized horse's serum that will protect a guinea-pig weighing 350 grammes against 1000 times the fatal dose of tetanus toxin. The serum having been standardized, it is stored in syringes convenient for therapeutic use. As in the case of diphtheria antitoxin, antitetanic serum loses its potency after a variable length of time.

Therapeutic Use of Antitetanic Serum.—The therapeutic efficiency of antitetanic serum depends chiefly on whether it is able to reach and neutralize the toxin before the latter becomes fixed by the cells of the central nervous system. Failure of tetanus antitoxin to have therapeutic effects is due in most cases to faulty administration. Ashhurst and John in a recent article (*Am. Jour. Med. Sc.*, June and July, 1918) present what is undoubtedly the most able and comprehensive view of the whole subject of the treatment of tetanus. Their recommendations will be followed here to a great extent.

Antitetanic serum is used both as a preventive and a curative measure. For prophylactic use, in the case of all wounds in which the development of tetanus is feared, at least 1500 units of the antitoxin should be injected as early as possible into the muscles or if possible into the nerves in the immediate neighborhood of the wound. The longer the interval that is allowed

to elapse between receipt of the wound and injection of the serum, the larger the dose of antitoxin required to be efficient. Owing to the fact that the antitoxin is all eliminated from the system in eight or ten days after the injection, and that dormant tetanus spores in the wound may develop after that time, it is advisable to repeat the injection during the second week, and if possible again during the third week. In the prophylaxis of tetanus, however, removal of any tetanus bacilli that may be present by thorough opening and disinfection of the wound is of greater importance than administration of antitoxin.

Antitetanic Serum as a Curative Measure.—The efficiency of tetanus antitoxin as a curative agent depends on three factors: (1) the site of the injection, (2) the frequency of the injection, (3) the quantity of antitoxin injected.

(1) *The Site of the Injection.*—Of all methods, the subcutaneous inoculation of tetanus antitoxin is least efficient, because, as Ashhurst and John point out, “by this method only a homœopathic dose ultimately reaches the motor nerves through which the toxin is being carried to the spinal cord, while by far the greater part of the antitoxin is distributed to the viscera, where it can be of no possible use. Administered in this way, overwhelming amounts are required to produce any effect.” Clinical and experimental

evidence points overwhelmingly to intraspinal (subdural) and intraneural injections as the best methods of administering tetanus antitoxin, for by these means the tissues affected by the toxin are reached directly. Intravenous injection ranks next, as by this means all the spinal nerves are reached at once, though the antitoxin reaches them much diluted.

(2) *Frequency of Injection*.—The frequent failure of tetanus antitoxin to effect a cure is due in part to the fact that it is not given often enough. The antitoxin can be given subcutaneously every three hours, intravenously once or twice in the twenty-four hours, intraspinally and intraneurally every day if necessary.

(3) *Quantity of Antitoxin Injected*.—The sheet-anchor in treatment is to get the maximum quantity of antitoxin indicated into the patient's body as soon as possible. One of the authors (B. A. T.), in the successful treatment of a case of tetanus, reported in *Monthly Cyclopædia and Medical Bulletin*, June, 1911, administered 213,740 units of antitetanic serum. Of this enormous and unprecedented quantity, 15,840 units were injected intraspinally, the remainder subcutaneously. The largest inoculation at any one time was 35,400 units and the maximum quantity in twenty-four hours was 97,940 units. Subsequently, Ashhurst and John state that *subcutaneously*, in the usual acute type of case, at least 100,000 units

are required in the first twenty-four hours; *intravenously*, probably 15,000 to 25,000 units should be administered at first; *intraspinally*, from 8000 to 10,000 units should be given, according to the severity of the case. The writers mentioned recommend for *intraneural* injections as great amounts as the nerves will absorb, ranging from 750 to 1500 units. For intraspinal injection the same technic is employed as for lumbar puncture and spinal anæsthesia. For intraneural administration the motor nerve trunk supplying the region of the wound is exposed as near to the spinal cord as practicable, and the antitoxin slowly injected directly into the sheath.

We do not regard it as out of place here to quote in full Ashhurst and John's recommendations for the rational treatment of a case of tetanus:

The patient will be placed in quiet, with competent nursing facilities. As soon as possible after coming under observation, whether this be in the small hours of the night or at bright noon-tide, the motor nerves leading from the wounded part will be exposed, as near to the cord as practicable, and as much antitoxin as each will contain will be injected toward the spinal cord. An intraspinal injection of at least 3000 units will then be made according to the usual technic for spinal anæsthesia. If it is possible to prick the cord with the needle, so much the better. Next the wound of entrance of the infection will be widely opened, all foreign bodies, sloughs, etc., will be removed by forceps, scissors, or scalpel; the wound will be irrigated with hot peroxide of hydrogen, swabbed out with 3 per cent. alcoholic solution of iodine, and loosely filled with gauze soaked in the same solution, and injection of antitoxin will be made (1500 to 3000 units) deeply into the muscular tissues around the wound. Continuous proctoclysis, as used in cases of peritonitis, will be given; and by mouth or in the rectal fluid will be administered effective doses of chloral

and bromides, at appropriate intervals.¹ Feeding will be enforced, by the nasal tube passed under chloroform anaesthesia, if necessary. During the course of the first day a moderate amount of antitoxin will be administered intravenously; probably 10,000 units will suffice.

The intraneural and intraspinal injections of antitoxin will be repeated daily, under chloroform anaesthesia, until marked decrease in spasticity occurs. Every twelve hours, or less often, a moderate amount of antitoxin will be injected intravenously, or even subcutaneously, so as to neutralize the circulating toxins; but the main reliance will be placed on intraneural and intraspinal injections. The administration of spinal depressants will be continued as long as they are indicated; a comatose state or muscular relaxation naturally are contra-indications. The wound will be dressed daily, as above described, until a healthy granulating surface is obtained.

The administration of cardiac, pulmonary and renal stimulants to meet the particular conditions, is invariably a matter of necessity.

The application of the treatment as above outlined and commenced within twelve hours of the onset of symptoms, should reduce the mortality of tetanus to less than 20 per cent.

Antigonococcic Serum.—Attempts have been made by Torrey and others to produce an antitoxic serum by injecting a toxin separated by filtration from cultures of gonococci. But it has been found that while this substance is toxic for laboratory animals, it does not produce antibodies that render the animal in-

¹ The authors feel from their experience that complete reliance should not be placed on these drugs, and recommend intraspinal injections of chemically pure magnesium sulphate in 25 per cent. solution to allay muscular spasm and convulsive seizures, in quantities of 2 to 5 c.c., the maximum being 1 c.c. for each 25 pounds of body weight. Should signs of respiratory failure supervene, chlorotone administered by mouth or by rectum, preferably the latter in one drachm doses, may be substituted for or alternated with the injections of magnesium sulphate.

jected immune to the toxin. Efforts to produce immune sera by injections of gonococci themselves have been more successful, and will be discussed under antibacterial sera (Chapter VII).

Antituberculosis Serum.—Mariagliano has produced a serum by immunizing horses with toxins made from the filtrate of cultures of tubercle bacilli. His favorable results have not been generally confirmed, and antiserum is little used in tuberculosis.

Antidysenteric Serum.—The dysentery bacilli of the Shiga type form an extracellular toxin and from this it is possible to prepare a true antitoxic serum. The toxin, together with antidysenteric serum, is injected into the horse in gradually increasing doses. Killed cultures of the bacilli are also injected to give the serum antibacterial properties. The standard strength of the antiserum is such that 0.5 c.c. will protect a 1500-gramme rabbit against three times the smallest fatal dose of toxin (Schorer). In Japan by the use of this serum, the mortality of dysentery of the Shiga type has been reduced from 28–37 per cent. to 8–12 per cent. In mild cases one dose of 10 c.c. of serum is injected, in severer cases two injections of 10 c.c. are made six to ten hours apart, never more than 20 c.c. in one day. Dysentery caused by bacilli of the Shiga type is rare in the United States, being largely confined to tropical and semitropical coun-

tries. The form of bacillary dysentery met with in the United States is usually due to the mannite-fermenting or Flexner type of organism, which will be dealt with in the chapter on antibacterial sera.

Antibotulism Serum.—Meat poisoning is due in many cases to a toxin produced by the *Bacillus botulinus*. This organism is anaërobic, and depends for its action upon an extracellular toxin. This toxin can be obtained artificially from bouillon cultures of the organism. The Institute for Infectious Diseases in Berlin has produced an antitoxin by immunization of animals, but data are still insufficient to pass judgment upon its curative properties.

Antiphytotoxigenic Serum.—Many forms of hay fever are manifestations of anaphylaxis or hypersensibility to the pollen of certain plants, such as golden-rod, ragweed, honeysuckle, chrysanthemum, etc. Dunbar has produced an antitoxin, known as “pollantin,” by injecting horses with extracts from various pollens. The antitoxin is put up in liquid form for inoculations, in powder form for local application. In certain cases the antitoxin gives protection and relief, but as a rule only for a limited time. Its use is occasionally attended by severe symptoms of anaphylaxis, so that caution must be observed in beginning treatment with it.

Antivenin.—Antivenin is an antitoxic serum prepared to counteract the effects of snake poison. The

nature and mode of action of snake venoms have been studied extensively, especially by Calmette and Flexner and Noguchi. Their researches have shown that poisonous snakes can be classified into two broad groups, according to the effects produced by the toxins of their venom. To one group belong the cobras, whose venom is principally in the nature of a neurotoxin, acting especially on the respiratory centre in the medulla, while in the other group, containing the vipers and rattlesnakes, the venom contains a hemorrhagin, and causes extravasation of blood in various regions of the body. In addition, all snake venoms possess hæmolytic properties, found most markedly in cobra venom. Against the neurotoxin of cobra venom, Calmette has successfully produced an antitoxin (antivenin) by injecting horses with the venom. This is only of therapeutic value, however, against cobra bites, and is useless as an antidote for rattlesnake bites. Attempts have been made, but unsuccessfully so far, to produce a reliable antihemorrhagic serum for the treatment of rattlesnake bites.

The antivenin of Calmette may be injected in doses of 10 to 20 c.c. To be of any value it naturally must be used as soon as possible after the bite is received, and is probably useless after 3 or 4 hours. The serum has, therefore, only limited application as a therapeutic measure.

VII

ANTIBACTERIAL SERA

It has been experimentally possible to produce antibacterial sera for a much larger number of organisms than antitoxic sera, though in the case of none of them have such brilliant therapeutic results been obtained as in the case of diphtheria and tetanus antitoxin. Yet in infections by a few organisms, notably the staphylococcus, streptococcus, pneumococcus, gonococcus, meningococcus, typhoid bacillus, colon bacillus, dysentery bacillus, cholera vibrio, plague bacillus and anthrax bacillus, antibacterial sera of considerable therapeutic value have been produced.

Antistaphylococcic Serum.—Various attempts have been made on horses and other animals to produce a potent antistaphylococcic serum, but they have almost invariably resulted unsuccessfully, although Doyen and Paltchikowsky assert that they have succeeded partially. Schorer states that the value of the serum is inconsiderable, and its injection in the treatment of staphylococcus infections is seldom or never warranted. Such noted authorities as Ehrlich, Bordet and Citron omit even to mention, in their works on immunity, the existence of antistaphylococcic serum. The failure, or only partial success, in producing

potent antistaphylococcic serum has possibly been due to utilization of avirulent or univalent cultures of the organisms. One of the most important factors conducive to the successful production of this serum is the employment of staphylococcus cultures from many sources. With this essential in mind, one of us (Thomas) isolated eighteen strains of *Micrococcus aureus* in pure culture from many sources, as furuncles, carbuncles, abscesses of the scalp, thoracic empyema, axillary abscess, and septicæmia. From these strains 24-hour cultures were grown and from the mixed growth a suspension in physiologic salt solution was prepared containing 32,400,000,000 cocci to the cubic centimetre. This was then heated for one hour over a water-bath at 60° C. The animal selected for the purpose of immunization was a full-grown ram weighing 165 pounds. The degree of immunity of the animal was governed by determination of the opsonic index for the polyvalent staphylococcic suspension (see Fig. 3). At the first injection the ram received intraperitoneally 972,000,000,000 dead staphylococci. Thereafter at weekly intervals either gradually increasing doses, or slightly smaller doses heated for a shorter time, and finally unheated organisms, were injected. After twelve inoculations, the index was found to be 2.6, and the animal was bled to death from the carotid artery. The serum was hermetically

sealed in glass ampoules containing 1 and 2 c.c. each. The appended chart (Fig. 8) shows the inoculations and curve of opsonic indices illustrating the immunity of the ram.

From therapeutic employment of this antistaphylococcic serum in many cases of carbuncles and furuncles, in doses ranging from 1 to 6 c.c., the following conclusions were drawn.

1. The antistaphylococcic serum as herein prepared and described possessed unquestionable therapeutic efficiency in a series of conditions, both general and local, due to infections by the *Micrococcus aureus*.

2. Biologic therapy by a potent polyvalent antistaphylococcic serum is more effective in the presence of a staphylococcic bacteriæmia than is the corresponding autogenous bacterin.

3. By virtue of the more immediate and decisive effects of the antiserum, it deserves first choice over the bacterin in the treatment of furunculosis and carbunculosis; on the other hand, a more intensive and lasting immunity can be conferred on the individual by supplementing the serum with two or three inoculations of the autogenous bacterin.

4. It is to be regretted that no attempt was made to standardize this antiserum with respect to standard units, since it must be conceded that the therapeutic failure or inefficiency of many serums is referable to

the deficiency of the immune-body content of that particular serum, or in other words, to an improper or incomplete immunization of the animal utilized for the production of the antiserum.

Antistreptococcic Serum.—Various antistreptococcic sera have been employed for some time with more or less success. They are nearly all made by injecting horses with increasing doses of killed streptococci recovered from different lesions, thus securing polyvalent antisera. Standardization of the strength of these sera is difficult, the initial dose being more or less empirical, and succeeding doses being guided by clinical effects observed.

The serum is indicated in all streptococcic infections, particularly in septicæmia, where rapid effects are desirable, and where bacterial vaccines are not suitable. The dose varies from 10 to 100 c.c., to be repeated in accordance with the symptomatic indications.

Antipneumococcic Serum.—Passive immunization in pneumococcus infections has been attempted by means of antibacterial serum obtained from horses previously injected with different strains of pneumococci. This serum depends for its action chiefly upon an increased production of bactericidal and opsonizing substances. In pneumonia the results of its use by most observers have been disappointing, the dose of

serum ranging from 10 to 20 c.c. in this disease. Cole finds the results of treatment of pneumonia by anti-pneumococcic serum to be encouraging in certain cases, when used in large doses (100 c.c. or more). He states that this action is probably in part due to antitoxic substances. In other pneumococcus infections, especially serpiginous ulcer of the cornea, benefit from its use has been seen.

Antigonococcic Serum.—Torrey and Rogers have produced an antibacterial serum that is of considerable therapeutic value in certain gonococcus infections. This serum is made by injecting strong, full-grown rams with 24-hour polyvalent cultures of virulent gonococci. Nine or ten injections are usually required to produce a serum of high potency, the bacterial suspension being heated for half an hour at 65° C. before the first two or three injections, after which the unheated gonococci are used. When sufficiently immunized the animal is bled from the carotid artery, the serum separated, and stored in sterile ampoules. Standardization of this serum in units is difficult, but some idea of its strength may be gained by testing its agglutinating power. The dose of the antigenococcic serum varies from 2 to 6 c.c., injected under the skin, and repeated every few days. The serum is only to be used as an adjunct to other methods of treatment. It is of little or no value in acute and chronic urethritis,

prostatitis, and conjunctivitis, but has beneficial effects in cases of gonorrhœal arthritis, endocarditis, peritonitis, and septicæmia.

Antimeningococcic Serum.—Kolle and Wassermann, Jochman, and others have succeeded in immunizing animals by injections of the *Diplococcus intracellularis* of Weichselbaum, the antisera thus obtained proving of considerable value in meningitis due to this organism. The serum of Flexner and Jobling, however, both on account of its potency and also the method of its administration, has superseded all others. Flexner and Jobling prepare their serum by injecting into horses, first, gradually increasing doses of heated meningococci, followed by injections of unheated organisms, and finally injections of an autolysate or extract of the meningococci which contains the endotoxin. The serum thus produced acts both by opsonizing and bacteriolytic properties. Accurate standardization of the serum is not possible, the dose depending largely upon clinical indications. Flexner and Jobling administer the serum by subdural injection, a lumbar puncture being first done and some of the spinal fluid removed. The quantity of serum to be injected ranges from 30 to 60 c.c. In severe cases the injection must be repeated in 12 or 24 hours and thereafter as long as meningococci are found in the spinal fluid. By means of this serum, the mortality of epi-

demic cerebrospinal meningitis due to the *Diplococcus intracellularis* has been reduced from 70 per cent. to about 80 per cent.

The antimeningococcic serum may also be used as a preventive measure in persons exposed to the disease.

Antityphoid Serum.—Antibacterial sera have been prepared by immunizing horses by injections of mixed strains of killed and live typhoid bacilli. The serum thus produced has been injected into persons suffering from typhoid fever, in daily doses of 10 c.c. or more. So far the results have been disappointing either in shortening the disease or lessening the severity of the symptoms. For protective purposes, active immunization by injection of killed cultures of typhoid bacilli has proved more successful.

Anticolonic Serum.—Hans Much finds that normal blood-serum has a bactericidal action upon certain strains of colon bacilli, while on other strains blood-plasma has this action but not blood-serum. He has employed normal blood-plasma and serum locally in cystitis caused by infection with the colon bacillus with some success. Much has also been able to produce a powerful specific antiserum by injecting animals with several strains of colon bacilli, which have bacteriolytic properties against all strains of colon bacilli. He has used this also with benefit as a local application in colon bacillus pyelitis and cystitis.

Antidysenteric Serum.—We saw that an antitoxic serum has been successfully produced and used in infections with the Shiga type of dysentery bacillus. The form of dysentery more common in the United States is due to the mannite-fermenting or Flexner type of organism. An antibacterial serum has been obtained for treatment of infection with this organism, but used with indifferent success.

Anticholera Serum.—Attempts have been made to obtain both antitoxic and antibacterial sera for use in cholera. Kolle and Wassermann, Metchnikoff and Roux tried to get a soluble toxin from cultures of the vibrio, and from that to make an antitoxin. But it is probable that they only obtained the intracellular toxin from disintegration of the organisms. The sera produced by inoculation of animals with these endotoxins or with the bacteria themselves, have been of little value in the treatment of cholera. More favorable results have followed active immunization with cultures of the organisms.

Antiplague Serum.—Two types of antiplague serum have been employed. The first is made by inoculation of horses with plague bacilli, and the other by inoculation with nucleo-proteids produced from cultures. Extensive trials of these sera in India show little benefit from their use in the treatment of plague, though life may be slightly prolonged by them.

Anti-anthrax Serum.—Of the many antisera that have been produced for the treatment of anthrax, that of Sclavo is the best. This is prepared by inoculation of asses simultaneously with cultures of the *Bacillus anthracis* and antiserum, a very powerful serum being thus obtained. Sclavo advises a dose of 80 to 40 c.c. divided into three or four parts, injected into the abdominal wall in different regions. This dose may be repeated in 24 hours if necessary. In grave cases, intravenous injection of the serum is recommended. Statistics tend to show that use of the serum reduces the mortality of anthrax from about 26 per cent. to 6 per cent.

Antimelitensis Serum.—A curative serum for the treatment of Malta or Mediterranean fever was first prepared by Wright by immunizing goats with cultures of *M. melitensis*. Horses were later employed for immunization with more or less encouraging results. However, the method of passive immunization in this disease has been overshadowed in recent years by active immunization.

VIII

MISCELLANEOUS SERA AND EXTRACTS

ANTIRABIC SERUM—ANTILEPROSY SERUM—ANTITYPHOID
EXTRACT OF JEZ—LEUCOCYTIC EXTRACT—ANTIFER-
MENT—ANTICARCINOMATOUS EXTRACTS—PYOCYA-
NASE—ANTITHYROID SERUM AND EXTRACTS—SPANG-
LER'S CROTALIN—PHYLACOGENS

THERE are several extracts and sera that have been introduced as methods of treatment in various diseases, but which require little more than brief mention, either because they have not yet been given thorough trial, or because results with them have not been sufficiently striking as to establish them as routine measures.

Antirabic Serum.—The success of active immunization in the prevention of hydrophobia has led several workers to attempt to obtain a serum for passive immunization in this disease. Such immunity has been experimentally produced by means of serum from dogs that have been actively immunized by injections of rabies virus. The practical value of the serum, however, is not yet sufficiently established. The recent announcement of the isolation and artificial culture of the rabies organism by Noguchi leads to the hope of success along this line.

Antileprosy Serum.—As yet, it has not been possible to cultivate the leprosy bacillus on artificial

media, a fact that has retarded considerably any research on immunization in leprosy. Passive immunization has been attempted by Carrasquilla, Herman and Abraham, and others, by injecting the blood of lepers or the juice from nodular lesions into the horse, and employing the serum from the animal thus injected in the treatment of the disease. Slight improvement has been reported in a few cases.

Antityphoid Extract of Jez.—Jez claimed antitoxic properties from an extract prepared from the bone-marrow, spleen, and lymph-nodes of an animal immunized to typhoid bacilli. This has been found to have but little value in the treatment of typhoid fever.

Leucocytic Extract.—Attempts have been made to increase the phagocytic powers by preparing and injecting extracts made from leucocytes, especially in pneumonia. Both human and lower animal extracts have been employed. Manoukhine uses leucocytes from the patient's own blood, removing them from 7 c.c. of the blood by centrifuging. He kills the leucocytes by freezing, and then suspends them in 1 c.c. of salt solution, which he injects. He claims to have obtained beneficial results in pneumonia by this method.

Antiferment.—The antiferment treatment of infections was introduced by A. Müller in 1907. In the destruction of the polymorphonuclear leucocytes dur-

ing suppuration there is liberated from them a proteolytic ferment known as proteolysin which has the power of dissolving the albumin of the tissues and thus breaking down the limiting wall of the abscess and allowing it to extend. Theoretically, therefore, much good would be accomplished if this tissue destruction could be inhibited. Normal blood-serum contains *antiferments* which neutralize the proteolysis of the leucocytes, but this under most conditions of inflammation is unable in sufficient quantity to reach the tissue that is being broken down. Müller therefore recommended the direct introduction of the anti-ferment in large amount by injecting normal serum into the inflamed area, or by packing abscess cavities, after evacuation of the pus, with gauze saturated with the serum. The patient's own blood-serum or ascitic or hydrocele fluid is recommended for the purpose. Where it is impractical to obtain these, normal horse serum can be substituted. While this form of treatment has some theoretical justification, practically it has not met with any notable success. The injection of acute abscesses is not unattended with danger of anaphylaxis. Irrigation of abscess cavities or packing with gauze saturated with the fluid may in some cases be a useful adjunct to the ordinary surgical measures.

Anticarcinomatous Extracts.—Extracts have been prepared from various normal and abnormal animal tissues for use in the treatment of cancer. While certain of these gave great promise at first and apparently brought about cures, none of them so far have stood the test of time. Again, certain substances that have given successful results in the cure of the artificially implanted malignant tumors in lower animals, have not had the same effects in corresponding disease in man. Among non-specific products that have been tried for this purpose may be mentioned extracts of thymus and thyroid glands, spleen, and pancreas. Coca and Gilman prepared emulsions from human carcinomata and injected them into patients suffering from growths of similar histology. This method was hailed at first as the long-looked-for specific non-surgical treatment for cancer, but has not lived up to expectations. The injection was supposed to stimulate the formation in the patient's blood of substances capable of dissolving the tumor tissue. Recognizing that malignant tumors are analogous in histological and physiological characteristics to embryonic tissues, Fichera (*Lancet*, Oct., 1911) announced the results of his experiments with autolysates of fetal tissues in patients suffering from carcinoma. Of 36 patients, in 18 the results were inconclusive, in 8 there was no

marked benefit, in 5 there was distinct benefit, while 5 were apparently cured. The autolysates were made by mincing fetal and embryonic tissues, placing them in normal salt solution and incubating for two months. The clear fluid was then injected subcutaneously or directly into the neoplasm. Recently, Babcock (*International Clinics*, Vol. II, 23rd Series, 1918) has reported a trial of fetal autolysates prepared by Fichera's method in 21 cases of recurrent or inoperable cancer, and concludes that this method is of no distinct value in the treatment of malignant disease in man. It would seem, therefore, that Fichera's treatment is doomed to the same failure that has so far attended all other attempts at cure of carcinoma by organo- or serum therapy.

Pyocyanase.—Pyocyanase is a ferment isolated from cultures of *B. pyocyaneus*, said to have high digestive powers for proteids. It has been employed in the treatment of diphtheria, the protein digestive action facilitating the removal of the membrane. Pyocyanase has also been used in gonorrhœal urethritis, in follicular tonsillitis and as a spray for disinfection of the nose.

Antithyroid Serum and Extracts.—Working upon the established theory that the symptoms of exophthalmic goitre were due to a hypersecretion of the

thyroid gland, investigators have attempted to formulate a method of treatment upon rational lines of organotherapy. In 1899 Otto Long employed the milk of thyroidectomized goats, believing that this possessed some property antagonistic to the gland secretion. Under the name of rodagen, a powder was prepared from the milk of thyroidectomized goats, 50 per cent. of which was composed of the active constituent of the milk and 50 per cent. sugar of milk. The dose of this is 1 to 3 drachms a day. Considerable improvement was noted following its use in some cases, but no disappearance of the exophthalmos, the goitre, or the tachycardia. Others have employed antithyroid serum from the blood of thyroidless sheep in doses of 1 to 5 c.c. daily. This had no conspicuous effect. Thyroidectin, a powder consisting of the desiccated blood of thyroidectomized animals, has also been used, it is claimed, with marked benefit in some cases of exophthalmic goitre. The dose is one or two 5-grain capsules three times a day. None of these preparations can be said to have fulfilled expectations.

Crotalin in Epilepsy.—The apparent improvement in an epileptic after being bitten by a rattlesnake led Spangler to treat a number of epileptics by injections of increasing doses of rattlesnake poison or crotalin. Spangler reported a certain number of favora-

ble results, which, however, have not been confirmed by others. The irrational basis for this treatment and the dangerous nature of the poison are such as to make its use unjustifiable. Among others, Yawger (*Penna. Med. Jour.*, Sept., 1914, p. 964), after a trial of crotalin in six cases of idiopathic epilepsy, gives the results of his experience as follows: "Two patients were uninfluenced; two were worse during the treatment; one, early in the course, developed such intolerant toxic symptoms that further experimentation was unjustified, and the last patient died two and a half months after treatment. While we did not feel that death resulted from the use of crotalin, the patient's disease certainly was not benefited by the treatment."

Phylacogens.—Within the last year or two considerable exploitation has been given to certain bacterial products for the treatment of infections of all kinds, under the name of *phylacogens*. It is claimed by the manufacturers that these "modified bacterial derivatives" are superior to bacterial vaccines as ordinarily prepared. Phylacogens are sterile aqueous solutions of bacterial derivatives prepared by growing the bacteria in artificial culture media, killing them by heat, and then removing their soluble products by filtration. In preparing special phylacogens for the

treatment of various diseases, the mixed products of several varieties of bacteria are employed, whether these varieties of bacteria are found in the particular infection or not.

Without going into further detail, we are of the opinion that the therapeutic use of phylacogens is based upon erroneous conceptions of infection and immunity, is unscientific, empirical, and unjustified. Moreover, there is evidence that their administration is not unattended with risk to the patient.

IX

AGGLUTININS

THE WIDAL PHENOMENON AND OTHER AGGLUTINATION REACTIONS

AGGLUTININS are antibodies belonging to the second order of Ehrlich (see Chapter III). They are formed in the blood-serum as a result of infection with specific bacteria. Agglutinins have the power of causing the specific bacteria, in response to which they have been called forth, to lose their motility and to be drawn together in clumps. Agglutinins are probably not very important factors in immunity, but are of great value for diagnostic purposes, as their action can be studied *in vitro*. Thus blood-serum in various dilutions, when brought in contact with a culture of the particular organism that has infected the patient, will after a time cause loss of motility and clumping of the bacteria in the culture. The principal infections in which agglutination tests have proved most serviceable in diagnosis are those caused by typhoid, paratyphoid, dysentery, and colon bacilli. These tests are of great value, sometimes in differentiating closely related organisms. Agglutinins belong to what are known as "group-reaction" antibodies, that is to say, they act upon closely allied

members of the same group of bacteria, such as *B. typhosus*, and *B. paratyphosus A* and *B*, though not with the same intensity. Members of this group can be differentiated by using different dilutions of the agglutinating serum, the specific organism involved being agglutinated by the greatest dilution of serum. Agglutination tests are occasionally employed in the diagnosis of glanders, cholera, Malta fever, and in staphylococcus and streptococcus infections.

THE WIDAL REACTION

The agglutination test for the diagnosis of typhoid fever is known generally as the *Widal reaction*, because this investigator, though by no means the discoverer of the method, first used it extensively in clinical work.

There are two methods of carrying out the test: microscopically and macroscopically. Of these, the microscopic method is that more generally employed.

A. MICROSCOPIC METHOD

For the performance of the test we require blood-serum from the suspected patient, and a recent culture of typhoid bacilli.

The description of the test may therefore be divided into three portions, viz.: (a) collection and dilution of the patient's blood-serum; (b) prepara-

tion of the culture of typhoid bacilli; (c) technic of the test.

(a) *Collection and Dilution of the Patient's Serum.*—There are several methods of collecting blood from the patient, which will be given in the order preferred.

(1) By means of Wright's capsule: A piece of $\frac{1}{4}$ -inch glass tubing is drawn out to a fine capillary stem at each end, leaving a portion with the original calibre about 2 inches in length. One of the capillary ends of the tube should be bent (see Fig. 22, B). The



FIG. 4.—Capillary test pipette for removal of serum from clotted specimen of blood.

patient's blood is drawn into the curved end of the capsule by capillary attraction from a puncture of the finger or lobe of the ear. When the tube is almost full, the ends can be sealed in a small flame. After the blood has clotted and the serum separated, a file mark is made at a convenient level on the tube, which is then broken, and the clear serum drawn off by means of a capillary pipette (Fig. 4). Any desired dilution of the serum can now readily be made by taking one drop of serum from the pipette, and the required number of drops of distilled water with the same pipette. The dilutions usually employed in performing the Widal

reaction are 1 in 40 and 1 in 80. The dilutions are conveniently made in small test-tubes or watch-glasses. For the 1 in 40 dilution, one drop of serum is placed in the test-tube or watch-glass by means of the pipette, and 19 drops of distilled water are then added with the same pipette. This makes a 1 in 20 dilution of the serum, a platinum loopful of which added to a loopful of bouillon typhoid culture gives the desired 1 in 40 dilution. Similarly for the 1 in 80 dilution, one drop of serum and 39 drops of water are mixed in a test-tube, making a dilution of 1 in 40. A loopful of this together with a loopful of typhoid culture gives the 1 in 80 dilution. This is the most reliable method of preparing the patient's serum, as it is free from red cells, and the dilution is accurate.

(2) By means of a leucocyte counting pipette: The blood is drawn up into the leucocyte counting pipette of the Thoma-Zeiss apparatus, as far as the 0.5 mark, just as for a leucocytic count. Instead of the acetic acid used in the latter procedure, however, the tube is filled up to the 11 mark with distilled water, thus immediately giving a dilution of 1 in 20. This is the most rapid method of preparing the patient's blood, and works very satisfactorily in hospital practice, where the test is usually carried out immediately after collection of the blood. The red cells interfere very little, if any, with the reaction.

(8) Employment of dried blood: Two or three drops of patient's blood are collected upon a clean glass slide or non-absorbent paper and allowed to dry there. In making the dilution, enough water is added to the dried blood to replace that lost in drying, and the clot well broken up in it by means of a platinum needle. The dilution can then be made by drops from a capillary pipette as described above. This method is useful in cases where the necessary apparatus for either of the other two methods is not at hand, and where the blood has to be sent for examination a distance. While the matter of dilution by this means is largely guesswork, reliable results can be obtained from it, though if possible it is better to use one of the other methods above described.

(b) *Preparation of the Culture of Typhoid Bacilli.*—A stock agar culture of typhoid bacilli should be kept at hand, and should be renewed by transferring to a fresh agar tube every three or four months. For use in the test a bouillon culture is made from this. The bouillon culture should be 24 to 48 hours old, grown at room temperature. In such a culture the typhoid bacilli are found to be present in sufficient numbers, of large size, and very motile. A satisfactory bouillon culture can be kept constantly on hand by inoculating a fresh tube with the preceding one every 48 hours. If the bacilli die out or prove other-

wise unsatisfactory, a bouillon tube can be inoculated from the original agar culture. Before using a culture for the Widal test, the organisms should always be examined for motility.

(c) *Technic of the Widal Reaction.*—The microscopic test is usually carried out with a depressed hanging-drop slide. The edge of the depression is ringed with vaseline. Upon a clean cover-slip are placed one platinum loopful of the bouillon culture of typhoid bacilli, and one loopful of previously diluted 1 in 20 patient's serum, giving a dilution of 1 in 40. The slide is pressed down upon the cover-glass, and quickly inverted so that the hanging-drop remains in the centre of the depression. A similar slide is prepared with a dilution of 1 in 80, and a third slide with culture only, to serve as a control for the motility of the typhoid bacilli. Each slide is marked with the dilution and time of beginning the test by means of a grease pencil.¹ Each slide in turn is now placed under the microscope, the high-power dry objective ($\frac{1}{8}$ in.) being used. The typhoid bacilli will be seen to move in all directions through the field. In from $\frac{3}{4}$ to 1 hour in the case of a positive reaction, all the typhoid bacilli

¹ We have found that plain glass slides can be used with equal satisfaction, as recommended by Stitt. A ring of vaseline is made on the centre of the slide, which is then gently allowed to rest on the cover-glass without any other pressure than its own weight. Sufficient space is thus preserved to allow of motility of the organisms.

in dilution 1 in 40 and probably also in 1 in 80 will have lost their motility, and most of them will be collected together in clumps throughout the field (Fig. 5). In the control slide, containing no serum, the organisms at the end of this time will still retain their motility, and there will be no clumping (Fig. 6). In case of a negative reaction, the bacteria in all the slides remain motile. If the slide in which a positive reaction

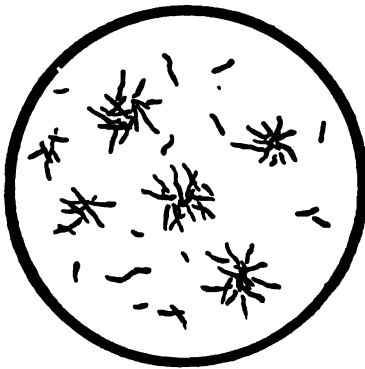


FIG. 5.—Widal's test, positive (agglutination or clumping).

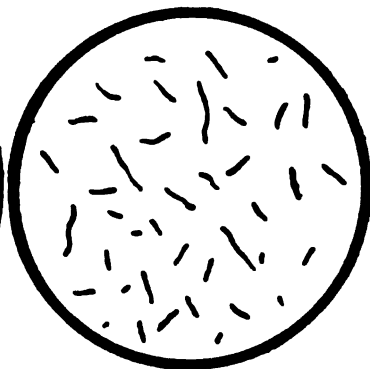


FIG. 6.—Widal's test, negative.

is taking place be observed from time to time, at first a few of the bacteria will be seen to lose their motility, and one will become attached to another, giving a V- or Y-shaped appearance. These groups will gradually be added to by other bacilli until clumps are formed and all motility is lost. Various grades of intensity of reaction are seen, ranging from formation of a few clumps and many organisms retaining their

motility to complete clumping and loss of motility of all. The degree of clumping should be reported as a partial reaction, which, although suggestive, is not necessarily specific for typhoid fever. Various grades of intensity are sometimes seen in the different dilutions. A positive reaction with 1 in 40 dilution may practically always be regarded as specific for typhoid fever, though occasionally blood of patients infected with *B. paratyphosus A* or *B* may give a positive reaction in this dilution with typhoid bacilli. A positive result with 1 in 80 dilution renders the diagnosis doubly sure.

B. MACROSCOPIC METHOD OF PERFORMING THE WIDAL REACTION

For this method small test-tubes, such as are employed in the Wassermann reaction, are used. Serum in different dilutions is placed in the tubes by means of small pipettes, and quantities of bouillon culture of typhoid bacilli are then added. A control tube consisting of the culture alone is also used. At the end of 12 to 24 hours at 37° C., in the case of a positive reaction, the bacilli become clumped and fall as a sediment to the bottom of the tube, leaving a clear fluid above. In the case of a negative reaction and in the control tube, the fluid remains uniformly cloudy. The reaction can be studied best by means of a reading glass. In this method killed cultures of the typhoid

bacilli may be employed, and have the advantage of keeping in good condition for some time. With ordinary laboratory facilities, however, the microscopic method is preferable.

OCCURRENCE AND SIGNIFICANCE OF THE WIDAL REACTION

The Widal reaction is usually not seen in typhoid fever until the second week of the disease, as some time is required for the production of the specific agglutinins in the patient's serum, upon which the test depends. The reaction usually persists for several months after recovery from typhoid fever, and persons have been known to react positively for years after the infection. Artificial immunization with typhoid vaccine also gives rise to a positive reaction which persists for some time. These facts should be remembered in weighing the results of the test performed in suspected cases of the disease. The diagnosis of typhoid fever should not rest upon the agglutination reaction alone. Other symptoms must be considered along with the laboratory test. The reaction may fail to appear in a small percentage of cases of undoubted typhoid fever, and may be positive occasionally in other conditions, particularly in jaundice. Probably the range of error on either side does not exceed 8 per cent., so that in the Widal reaction we have one of the most important cardinal signs of typhoid fever.

OTHER APPLICATIONS OF THE AGGLUTINATION PHENOMENON

For the identification of certain species of bacteria, the agglutination reaction is of the greatest value. For this purpose, agglutinins are artificially produced by injection of animals, usually rabbits, with cultures of the various organisms whose presence is suspected in given cases. Thus we have conditions reversed from those obtaining in the Widal reaction for typhoid fever, the serum being the known factor, and the organism the unknown. For example, we have isolated an organism that we strongly suspect to be the typhoid bacillus. If we find that a culture of this organism is agglutinated by the greatly diluted serum from an animal previously immunized to typhoid bacilli, and is not agglutinated by similar dilutions of sera from animals immunized to other organisms, such as *B. paratyphosus*, *dysenteriae*, etc., our suspicion is converted into certainty. This method is of great value, especially in the detection of typhoid bacilli in milk and other fluids, as well as in other procedures.

X

PRECIPITINS

SIGNIFICANCE AND APPLICATION OF PRECIPITINS— TECHNIC OF REACTION—SPECIFIC IDENTIFICATION OF BLOOD AND OTHER PROTEINS

WHEN a protein is brought in relation, *in vitro*, with blood-serum of an animal which has been immunized against that particular protein, the protein is thrown out of solution, the fluid becomes cloudy, and a sediment finally settles to the bottom of the tube. This is known as the *precipitation phenomenon*, and is due to the presence in the blood-serum of substances known as *precipitins*. Precipitins are antibodies formed in the blood in response to injection of unorganized protein material. They are antibodies of the second order of Ehrlich, and are analogous to agglutinins, which act upon organized proteins or bacteria.

The precipitation phenomenon is made use of in the identification of various proteins. Each species of animal has a specific protein, which can be detected by the precipitation reaction. Practically, the test has been made use of chiefly in (I) detection of adulteration of meat products such as sausages, etc.; and (II) in the medicolegal identification of blood.

(I) DETECTION OF ADULTERATION OF MEAT PRODUCTS

This test can be applied to determine whether sausages represented as being made purely of beef or

pork, contain horse meat. Preparation for the test consists in the immunization of animals, of which rabbits are the most suitable, by injections of extracts made from the various meats to be tested for, as horse, beef, pork, etc., each of which has a specific protein. The immune serum, on being brought in contact with an extract of the particular meat whose protein is specific for it, will bring about precipitation.

(II) MEDICOLEGAL IDENTIFICATION OF BLOOD

This test occasionally assumes the utmost importance when the question arises in murder cases as to whether blood stains are human or not. The chemical tests for blood do not distinguish one species from another, while microscopical examination of dried blood is unsatisfactory. Dried blood in very small quantities, however, will respond to the precipitation reaction, and by this means we can differentiate human blood from that of the lower animals. Uhlenhuth, Wassermann, and Schültz were the first to make use of the precipitation test in this connection.

THE PRECIPITIN REACTION

The technic of the reaction is divided into two parts.

(a) *Production of the Precipitating Serum.*—For this purpose rabbits are employed. Three or four in-

travenous injections of human blood-serum are made into the rabbit in quantities of 1-2 c.c. every five or six days. The strength of the antihuman serum thus formed may be tested after three or four injections. This is obtained by piercing a vein in the ear of the rabbit, collecting a few cubic centimetres of blood, allowing the clear serum to separate, and titrating it with different dilutions of human serum. The dilutions are to be made with normal salt solution. The antiserum may be regarded as of sufficient strength when 0.1 c.c. will produce distinct cloudiness when mixed with 1 c.c. of human serum in 1-1000 dilution after incubation for five minutes at 37° C. The antiserum, moreover, must give no cloudiness with plain salt solution, nor with the serum of any other animal in 1-200 dilution after half an hour at 37° C. The following table illustrates the titration of a suitable anti-human serum:

	Antihuman rabbit serum	Human serum 1 c.c.	Controls	Result after incubation for $\frac{1}{2}$ hour at 37° C.
	c.c.			
1	0.1	1-10	Marked turbidity
2	0.1	1-100	Marked turbidity
3	0.1	1-1000	Marked turbidity
4	0.1	1-5000	Turbidity
5	0.1	1-10000	Slight turbidity
6	0.1	1 c.c. normal salt solution	Clear
7	0.1	1 c.c. normal guinea-pig serum (1-200)	Clear

(b) *Identification of Blood.*—An extract of the blood clot is made with normal salt solution. This is then filtered until absolutely clear, and diluted until we have approximately a 1-1000 solution of the serum. The latter point can be determined by boiling a portion of the solution, as a 1-1000 dilution will then show a slight haziness, while greater dilutions remain clear.

One-tenth c.c. of the antihuman rabbit's serum is then mixed with 1 c.c. of the suspected serum thus prepared, and incubated for half an hour at 37° C. In another tube 0.1 c.c. of *normal* rabbit serum is placed with 1 c.c. of the suspected serum. In a third tube is placed 0.1 c.c. of antihuman rabbit serum and 1 c.c. of normal salt solution. The results in the case of a positive reaction are shown in the following table:

	Antihuman rabbit serum	Suspected blood	Normal salt solution	Normal rabbit serum	Result, 37° C.	
					After 5 minutes	After half hour
1	c.c. 0.1	c.c. 1 (1-1000)	c.c.	Turbidity	Marked turbidity
2	0.1	1	1	...	Negative	Negative
3	...	1 (1-1000)	..	0.1	Negative	Negative

It has not been found possible to employ this test for differentiation of the blood of various races of human beings, such as negro from Caucasian, etc.

The same test can be used for the blood of various lower animals by preparing suitable antisera. Closely related species, however, such as hens and pigeons, are not differentiated by it.

XI

LYSINS

BACTERIOLYSINS AND CYTOLYSINS (HÆMOLYSINS)

AMONG specific antibodies of the third order of Ehrlich we find *bacteriolysins*, which are substances acquired by the blood-serum of an animal that is immunized to specific bacteria. Bacteriolysins have the property of dissolving the particular bacteria for which they are specific. Analogous substances are likewise formed against body-cells known as *cytolysins*. Cytolysins that act upon red blood-corpuscles are called *hæmolysins*. In order for the phenomenon of bacteriolysis to take place, in addition to the specific bacteriolysin (also known as *amboceptor*), the presence of a second or non-specific substance is necessary. This substance is known as a *complement*, and is present in varying amount in the blood-serum of all animals whether immunized or not. The same complement, being non-specific, may take part in any bacteriolytic or cytolytic reaction. Amboceptors are said to be *thermostable*, i.e., they are not destroyed by heating to 56° C., while complement is *thermolabile*, and is destroyed at a temperature of 56° C. The non-specific element or complement can therefore be re-

moved from a serum by heating the latter to this temperature, rendering the serum inactive although it contains the specific element or amboceptor. The serum can be made active again by adding more complement in the form of fresh unheated normal serum. This discovery that two elements, one specific and the other non-specific, are necessary for bacteriolysis, was made by Bordet.

Pfeiffer's Phenomenon.—Our knowledge of bacteriolysins is dependent largely upon the following experiment carried out by Pfeiffer. He injected a suspension of cholera spirilla into the peritoneal cavity of guinea-pigs that had been previously immunized to cholera spirilla, and also into non-immunized guinea-pigs. In the case of the non-immunized animals the bacteria increased in number until they destroyed the animals. In the case of the previously immunized guinea-pigs, examination of the peritoneal fluid from time to time showed that the bacteria quickly died, became clumped together in granules, became broken up, and finally were dissolved. Practically, this phenomenon may be made use of in the identification both of bacteria and of specific immune sera. It is especially of value in the identification of cholera spirilla. For this purpose, guinea-pigs previously immunized to cholera are injected intraperitoneally with the suspected organisms, and the peritoneal fluid examined from

time to time for the occurrence of bacteriolysis. Passively immunized animals may also be employed for the observation of Pfeiffer's phenomenon. That is, the reaction will occur on injection of a mixture of cholera immune serum and a culture of cholera spirilla into the guinea-pig. This phenomenon can also be observed *in vitro*, by mixing immune serum and bacteria in a test-tube, and incubating at 37° C. for some time.

What has been said of bacteriolysins regarding the manner in which they operate, applies likewise to cytolytins and hæmolysins. Thus, for example, if a specific immune serum, which has been prepared by injecting the blood-corpuscles of a sheep into the peritoneal cavity of a rabbit, is mixed with the corpuscles of the sheep, and incubated at 37° C., the sheep's corpuscles will after a short time be dissolved (hæmolysis). If the rabbit's serum be previously heated to 56° C. for half an hour, hæmolysis will not take place because the complement or non-specific factor in the reaction will have been destroyed. The immune serum, though it still retains its specific factor or hæmolysin (amboceptor), has been inactivated. The serum can be re-activated by the addition of fresh normal serum of any animal, thus supplying complement, after which the hæmolytic action will take place as before.

In the reaction by antibodies belonging to the third order of Ehrlich, whether they be bacteriolytic,

cytolytic, or hæmolytic, the substances acted upon (bacteria, blood-corpuscles, etc.) are known as *anti-gens*; the specific antibody (bacteriolysin, cytolysin, hæmolysin) is spoken of as *amboceptor*; while the non-specific factor in the reaction is known as *complement*.

Various diagnostic tests of the greatest practical importance have this reaction as a basis, and will receive consideration in the following chapter.

XII

FIXATION OF COMPLEMENT

PRINCIPLES OF THE REACTION—BORDET-GENGOU PHENOMENON—WASSERMANN-NEISSER-BRUCK MODIFICATION—TECHNIC OF THE WASSERMANN REACTION IN THE DIAGNOSIS OF SYPHILIS—MODIFICATIONS OF THE WASSERMANN REACTION—HECHT-WEINBERG MODIFICATION—CLINICAL APPLICATION OF THE WASSERMANN REACTION—EFFECTS OF TREATMENT ON THE WASSERMANN REACTION

IN the foregoing chapter we have seen that bacteriolysis, cytolysis, and hæmolysis take place by means of a specific substance known as amboceptor, and a non-specific substance called complement. This union of antigen, amboceptor, and complement is spoken of as *fixation or absorption of complement*. The exact nature of the reaction is not known. In order that the complement-fixation reaction be understood in its application to specific diagnosis, it is necessary to explain first in some detail the process of hæmolysis. When red blood-corpuscles remain in suspension in a fluid, the fluid has an opaque red color. If hæmolysis occurs, the hæmoglobin leaves the corpuscles, the fluid becomes transparent, and has a deep red color. If no hæmolysis occurs, the corpuscles gradually fall to the bottom of the tube, leaving clear, colorless fluid above. Hæmolysis can occur in a non-

specific manner, *i.e.*, without amboceptor and complement, through several agencies. Thus, if the corpuscles be placed in plain water, hæmolysis will occur, and likewise through the addition of various substances, such as snake venom, tetanus toxin, etc. Specific hæmolysis differs from non-specific in that it will take place in an isotonic fluid such as physiologic salt solution.

In hæmolysis there is no actual solution of the cell. The process consists rather in a disturbance of the osmotic equilibrium between the cell contents and the surrounding medium. The hæmolytic amboceptor combines with the stroma of the cell, increases the permeability of the latter, setting free the hæmoglobin into the surrounding medium. Bacteriolysis is dependent upon a similar mechanism, and differs from hæmolysis in that it is practically invisible to the naked eye.

The blood of most animals usually contains a certain amount of *natural* hæmolysin for the red corpuscles of other animals. Thus, human blood-serum possesses a considerable natural hæmolytic power for sheep's corpuscles. This normal hæmolytic power can be greatly increased artificially by injection of the blood-corpuscles of one animal into another, so that very high dilutions of the second animal's serum will hæmolyze the corpuscles of the first, in the presence of

suitable amounts of complement. In experimental and diagnostic work, the rabbit is the animal usually employed for the production of specific hæmolytic serum. By three or four intraperitoneal or intravenous injections of suitable quantities of washed sheep's corpuscles into the rabbit at intervals of 8 or 4 days, the rabbit's serum will become so highly immunized that it will, in 1-1000 or greater dilution, hæmolyze an equal quantity of 5 per cent. suspension of sheep's corpuscles in the presence of a sufficient amount of complement. It has been found that complement is present in varying amounts in the blood-serum of different animals, but that the serum of the guinea-pig is most constant in this respect. So in order to insure the presence of a definite amount of complement, the immune hæmolytic serum (rabbit) is heated at 56° C. to destroy the complement present, and a definite amount of fresh guinea-pig serum afterwards added. The technic of the preparation and titration of specific hæmolytic serum will be given under the description of the Wassermann reaction.

THE BORDET-GENGOU PHENOMENON

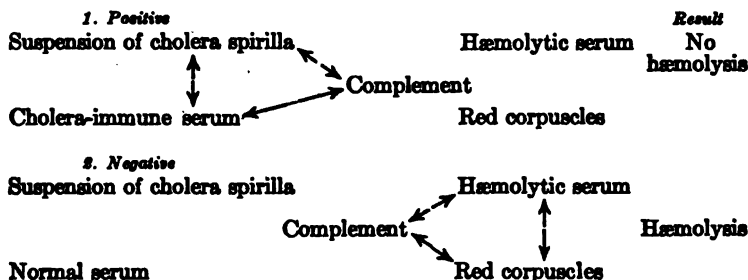
Bordet and Gengou were the first to employ the complement-fixation reaction in the diagnosis of specific infections. Since the phenomena of bacteriolysis and of hæmolysis each depend upon the presence and

fixation of a common non-specific substance termed complement, these observers conceived the idea of combining the two phenomena as a means of diagnosis. The components of the first-mentioned reaction, consisting of the bacterial suspension or *antigen*, the specific antibacterial serum or *amboceptor*, and *complement*, together form the *bacterial system*; the second part is known as the *hæmolytic system*, consisting of blood-corpuscles (*antigen*), specific hæmolytic serum (*amboceptor*), and *complement*. Bordet and Gengou took a suspension of cholera spirilla as antigen and mixed it with a certain quantity of cholera-immune serum made by injecting a rabbit with cholera spirilla, together with a definite amount of fresh guinea-pig serum as complement. This mixture was incubated at 37° C. for one hour. At the end of this time it was presumed that the interaction between antigen and amboceptor had taken place with *fixation or absorption of complement*. But since the reaction was not perceptible to the eye, a further procedure became necessary to show the result. For this purpose, a suspension of washed rabbit's blood-corpuscles and a quantity of hæmolytic serum for the rabbit's corpuscles were added, and the whole incubated for another hour. At the end of this time, it was found that no hæmolysis of the rabbit's corpuscles had taken place, because the complement had been fixed during the first incubation

by the bacterial system, none being left free to act in connection with the hæmolytic system.

In a second experiment, exactly the same components were used, except that the cholera-immune serum was replaced by normal serum. Hence, complement was not fixed by the bacterial system, but remained free to become a part of the hæmolytic system, and hæmolysis occurred.

The first or positive and the second or negative reactions may be graphically illustrated thus:



The specific complement-fixation reaction may be applied to the diagnosis of several diseases. By testing an unknown serum with a known bacterial antigen, it is thus possible to determine whether or not the individual furnishing the serum is infected by the particular organism from which the antigen is made. For some time after Bordet and Gengou's announcement, the reaction had chiefly a scientific rather than a practical interest. The first great advance toward rendering the method of practical importance was the dis-

covery by Wassermann and Bruck that extracts furnished by lysis of the bacteria will serve as antigens for the complement-fixation reaction equally as well as, if not better than, suspensions of the bacteria themselves. This fact was applied by the workers mentioned in the diagnosis of typhoid fever, tuberculosis, meningitis, etc.

THE WASSERMANN REACTION

A further step was now taken by Wassermann, Neisser, and Bruck. They recognized what an enormous value the reaction would have if it could be applied to syphilis, the causative organism of which had not been artificially cultivated up to that time (1906). So these observers conceived the idea of employing as antigen, extracts of organs known to be rich in *treponemata* (spirochætæ). From this the now universally used and important "Wassermann reaction" had its origin. In their first experiments Wassermann, Neisser, and Bruck made watery extracts from syphilitic fetal liver, in which *Treponemata pallida* are very abundant. Using these extracts they obtained fixation of complement with the serum of syphilitic individuals, and from their results concluded that the reaction was specific, just as that obtained in the case of typhoid bacillus extract and typhoid serum. But it was then found that alcoholic extracts of syphilitic tis-

sues would give the reaction in syphilis with equally good results. Since it is the *lipoid* material in tissue that is dissolved by alcohol, it followed that this lipid material must contain the substance that is antigenic for syphilitic serum.

It was then found by Marie, Levaditi, and Landsteiner that complement-fixation with syphilitic serum occurred when alcoholic extracts of *normal* tissues, such as liver, human heart, or guinea-pig heart, were used as antigen, and that the results of reactions with these extracts were as reliable as those where extracts of syphilitic tissues were used. These observations showed that the complement-fixation reaction in syphilis is not entirely a specific antigen-antibody reaction as in the case of those occurring between bacterial antigens and antibodies, but this fact in no way lessened the practical value of the test in the diagnosis of syphilis.

It has still further been shown that extracts prepared from lecithin, cholesterin, etc., can be used as antigens and some workers restrict themselves to these artificial products. The various forms of antigen in use at the present time account to a great extent for the divergence of results in some cases when serum from the same patient is examined by different workers. This has a tendency to throw doubt on the value of the reaction in the minds of skeptical clini-

cians. A great deal of work is being done upon the antigen question, and it is hoped that in the near future some definite standard will be adopted and universally employed. We believe, in the light of present knowledge, that in the Wassermann reaction there is probably a specific factor as well as a non-specific, certainly that the organisms of syphilis cause an overproduction of the special lipoid antigenic substance in the tissues, and that the most reliable results will be obtained by using an alcoholic extract of syphilitic tissue, in which this substance is found in greatest abundance. We adhere to this view as the result of the use of syphilitic liver-extract in several thousand tests, in which our results have differed in no material way from those of reliable workers.

We recently reported (*J. A. M. A.*, Jan. 31, 1914) the results of a trial of some cholesterinized extracts of guinea-pig heart and human heart. These extracts are made by adding cholesterin to the ordinary alcoholic extract of normal heart in the proportion of 0.4 per cent. The cholesterinized guinea-pig heart-extract was used by us along with our antigen of alcoholic extract of syphilitic liver in the routine examination of 100 cases, the cholesterinized human heart-extract being employed in thirty-three cases.

Careful preliminary titration of the antigens was carried out before doing the tests, and at no time was

more than one-third of the anticomplementary dose used. The comparative results of tests with syphilitic liver-extract and cholesterinized guinea-pig heart-extract may be divided into four groups as follows:

1. In twenty-two cases the results were positive with both antigens, with little or no difference in degree of reaction.

2. In eight cases the syphilitic liver-extract gave a weakly positive reaction, while the cholesterinized extract gave a stronger reaction.

3. In thirty-six cases both antigens gave a negative result.

4. In thirty-four cases the syphilitic liver-extract gave a negative reaction, while the cholesterinized heart-extract gave a weakly positive or medium positive result. This is naturally the most important group from the clinical point of view. Nine of these thirty-four cases were treated cases of syphilis exhibiting no symptoms, showing that it is more difficult to render the serum negative to the cholesterinized heart-extract by anti-syphilitic treatment. The remaining twenty-five of this group were cases submitted for diagnosis or which had no signs or history of syphilis. When one antigen reacted more strongly than the other, it was always the cholesterinized heart-extract. In no case did the syphilitic liver-extract give a stronger reaction than the cholesterinized extract.

In the comparative tests with the cholesterinized extract of human heart, of thirty-three cases there were six in the first group, that is, equally positive with the two extracts; four in the second group, in which the cholesterinized human heart-extract gave a distinctly stronger reaction than the syphilitic liver antigen; sixteen in the third group, in which both antigens gave negative results; seven in the fourth group, which were negative with the syphilitic antigen but positive with the cholesterinized heart-extract.

In the first series, therefore, the results with the two antigens disagreed in 42 out of 100 cases, and in the second series in 11 out of 33 cases. We see from these results that the cholesterinized extracts apparently give a more "delicate" reaction than the extract of syphilitic liver, but we also find that many weakly positive results are obtained by them in non-syphilitic cases. This fact alone is quite sufficient, in our opinion, to offset any advantage in delicacy of the reaction obtained with the use of cholesterinized extracts. We feel that just as high a percentage of positive results is obtained in known or clinically apparent syphilitics with the syphilitic liver-extract as is obtained with the cholesterinized antigens, and the former do not give positive results in non-syphilitic cases. The success in detecting mild degrees of syphilitic infection is doubtless due in part to the use of one

properly standardized unit of complement and hæmolytic amboceptor, as suggested to us by Laird, instead of double or triple units as employed by many workers. We must conclude that with cholesterinized antigens, varying degrees of inhibition of hæmolysis may be obtained in serums from many conditions other than syphilis, and in normal persons. While experimental investigations are to be highly commended, we therefore regard the employment of these artificial antigens for routine clinical use at the present stage of our knowledge, instead of being an advance in serologic technic, rather as a distinct step backward.

Schamberg, Kolmer, and others report that they obtained positive Wassermann reactions, using the cholesterinized antigens in over 28 per cent. of twenty-two cases of psoriasis, in a great many of which syphilis could almost certainly be excluded, thus providing evidence that weak reactions with these antigens do not necessarily mean syphilis, and that a diagnosis of syphilis cannot be based on weak and medium inhibitions when they are employed. We hold that weakly positive reactions with syphilitic liver-extract mean nothing but syphilis. Even though it were true that the cholesterinized antigens give a more "delicate" reaction and may furnish positive results in cases of syphilis that are negative to the syphilitic liver-extract, it is a very much less serious error to overlook an

occasional case of syphilis than to saddle a diagnosis of the disease with all it entails on a patient who does not have the disease.

Considerable harm is being done at present by the use of unreliable non-specific or artificial extracts, in two ways:

1. The marked discrepancies between the results of the Wassermann test and the clinical findings in many cases are causing skeptical clinicians to lose confidence in the value of the reaction, and thus they are being deprived of an important diagnostic and therapeutic aid.

2. A great many unfortunate persons are being treated for syphilis who have not and never had syphilis, as the result of weakly positive and doubtful reports of workers using these antigens.

Since Noguchi, in 1911, first cultivated the *Treponema pallidum* in a pure state, much work has been done by Noguchi, Kolmer, and others with antigens made from the treponemata themselves. The results have been disappointing for diagnosis of primary and early secondary syphilis, as only a relatively small proportion of these cases gives positive reactions when the spirochætal antigens are employed. But the positive reactions obtained in late secondary and tertiary cases do prove that there is a specific fixation of complement occurring when lipoidal substances are

brought in relation with syphilitic serum, but as yet we are in the dark in regard to the true nature of the phenomenon.

Several factors other than the antigen are important in obtaining reliable results with the Wassermann reaction; the most essential of which is the titration and use of minimal amounts of complement.

TECHNIC OF THE WASSERMANN REACTION

The following is a list of the apparatus, animals, etc., required, and has been made as complete as possible:

Electric centrifuge.	Normal salt solution (0.85 per cent.).
Several 15 c.c. graduated centrifuge tubes.	Sodium citrate solution (1 per cent. in normal salt solution).
Several 1 c.c. pipettes, graduated in tenths of a c.c.	Wire racks for holding small test-tubes.
Several 1.9 c.c. pipettes, graduated in hundredths of a c.c.	Platinum loop with glass handle.
Two 10 c.c. pipettes, graduated in tenths of a c.c.	Scissors, scalpel, dissecting forceps.
Capillary pipettes, prepared by drawing out 3/8 in. glass tubing.	2 c.c. all glass hypodermic syringe with small needle.
Rubber nipples for capillary pipettes.	Wax pencil.
100 1 c.c. glass ampoules.	95 per cent. alcohol, ether, xylol, glycerin.
100 small test-tubes, about 5 c.c. capacity.	Water-bath.
Several flasks, capacity 500 c.c.	Centigrade thermometer.
Two flasks, 100 c.c. capacity.	Incubator.
Two graduated 100 c.c. cylinders.	Bunsen burner.
Several Petri dishes.	Refrigerator.
	Sheep, rabbits, guinea-pigs.

All glass-ware and solutions mentioned above must be sterilized before using.

HÆMOLYTIC SYSTEM

We employ the anti-sheep hæmolytic system, *i.e.*, washed sheep's red blood-corpuscles and anti-sheep hæmolytic rabbit serum. As the hæmolytic amboceptor requires some time in preparation, its production will be described first. This anti-sheep hæmolytic serum—which hereafter for the sake of brevity will be termed *amboceptor*—is prepared by injecting quantities of washed sheep's red blood-corpuscles into a rabbit until in the presence of a suitable amount of complement the rabbit's serum in high dilution will dissolve corpuscles from the sheep when properly incubated *in vitro*.

Collection of Sheep's Blood.—The collection of blood and preparation of suspension or red corpuscles as described below is the same for the test itself as for injection into the rabbit. A sheep may be kept on the premises, or the blood may be obtained from a nearby slaughter-house. The latter if available saves a good deal of time. The sheep's blood can be drawn from the internal jugular vein by plunging a needle of large lumen into the vein and allowing the blood to fall into a flask containing pieces of glass or wire. The blood is defibrinated and thus prevented from clotting by shaking the flask immediately after collection of the blood. For the reaction, a 5 per cent. suspension of sheep's corpuscles is employed. Whole blood consists

approximately of one-half serum and one-half corpuscles. To make up a 5 per cent. suspension therefore, 1 c.c. of the defibrinated blood is placed in a graduated centrifuge tube by means of a 1 c.c. pipette, and the contents of the tube brought up to 10 c.c. by the addition of normal salt solution. This tube then will contain 0.5 c.c. or 5 per cent. of sheep's corpuscles. Several tubes can be prepared in the same way, to furnish sufficient suspension of corpuscles for the test.

A convenient method used by us for obtaining blood from the sheep is as follows: In several graduated centrifuge tubes are placed 9 c.c. of 1 per cent. sodium citrate solution in normal saline. The sheep's ear is washed off with alcohol, and one of the large veins severed with a scalpel (Fig. 7). One c.c. of the blood is now allowed to drop into each of the tubes, the sodium citrate preventing it from clotting. This gives a 5 per cent. suspension of sheep's corpuscles. It is necessary that all serum be removed from the suspension, in order to avoid anaphylactic symptoms if for injection into the rabbit, and to remove all complement if the corpuscles are to be used in the test itself. This is accomplished by centrifuging the tubes for 5 or 6 minutes in an electric centrifuge, at the end of which time the corpuscles will be found at the bottom. The volume of the corpuscles will vary according to the length of time used in centrifuging, the

longer the time the more closely will they be packed. From this it is easily seen that the method used of taking a definite amount of whole blood in the beginning and from that calculating the dilution required is more accurate than drawing out a quantity of the centrifuged corpuscles and making the dilution from them,



FIG. 7.—Method of obtaining blood from sheep's ear.

which is the method commonly employed. The clear fluid above the corpuscles is poured off, and replaced with normal salt solution, the tubes shaken, and again centrifuged. This washing and centrifuging is repeated three or four times, in order to remove all serum. If the corpuscle suspension is to be used in the test itself, the fluid in each tube is finally brought

up to its original volume with normal salt solution, making a 5 per cent. suspension. Practically, a larger amount of suspension can be prepared in the same number of centrifuge tubes, by originally placing say 8 c.c. of whole blood in each, washing and centrifuging three or four times, and after the final washing bringing the volume up to 30 c.c. For injection into the rabbit, a 10 per cent. suspension of the corpuscles is more suitable than a 5 per cent. suspension, so after the final washing the fluid in a tube containing originally 1 c.c. of whole blood or 0.5 c.c. of corpuscles is brought up to a total volume of only 5 c.c.

Immunization of the Rabbit to Sheep's Corpuscles.

—The suspension of sheep's corpuscles prepared as described above may be injected into the rabbit either intraperitoneally or intravenously. Since much larger amounts of suspension and a longer time for immunization are required by the former method, we have discarded it entirely in favor of the intravenous method. The latter is just as simple, and if proper precautions against infection and injection of air be taken, is quite safe. For the first injection about 1 c.c. of the 10 per cent. corpuscle suspension is drawn up into an all-glass hypodermic syringe of 2 c.c. capacity and fitted with the usual fine needle; the smaller the needle the better. The marginal vein of the rabbit's ear is made prominent by wiping it with

xylol, and the suspension slowly injected into it through the skin in the direction of the circulation (Fig. 8). Usually, to obtain a sufficiently powerful hæmolytic amboceptor, three injections at intervals of 3 or 4 days are required. The second injection should

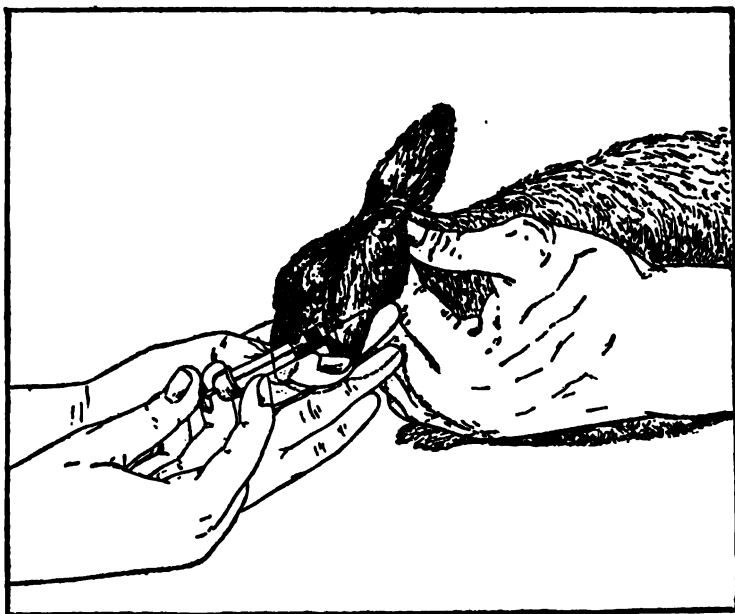


FIG. 8.—Showing method of intravenous injection or immunisation of rabbit with 10 per cent. suspension of sheep's blood-corpuscles for the production of hæmolytic amboceptor.

consist of 1.5 c.c. and the third of 2 c.c. of the suspension. Three or 4 days after the third injection, about 2 c.c. of the rabbit's blood is drawn from the ear by puncturing a vein, and the serum tested for its hæmolytic power against sheep's corpuscles. Thus in

ten days, or two weeks at the most, we are able to prepare a powerful hæmolytic amboceptor, which requires four or five weeks by the intraperitoneal method.

Titration of Hæmolytic Amboceptor.—For use in the Wassermann test we aim to produce a hæmolytic amboceptor of such strength that 1 c.c. of not less than a 1–2000 dilution of rabbit's serum will hæmolyze 1 c.c. of a 5 per cent. suspension of sheep's corpuscles in the presence of 0.1 c.c. of fresh guinea-pig serum used as complement. Usually, by the intravenous method described, the rabbit can be immunized so that its serum in 1–3000 dilution will fulfil these requirements. To determine the strength of the hæmolytic serum, three or four days after the third injection of the rabbit, about 2 c.c. of blood are drawn into a small test-tube from a vein of the rabbit's ear. When the blood has clotted and the clear serum separated, the latter is drawn off and the natural complement in it destroyed by heating the serum in a water-bath for half an hour at 56° C. This deprivation of complement is known as *inactivation*. The complement used in titrating the hæmolytic amboceptor and in the Wassermann test itself is furnished by serum of the guinea-pig, as it has been found that this animal has an almost constant amount of complement present in its blood. To obtain complement, a full-grown guinea-

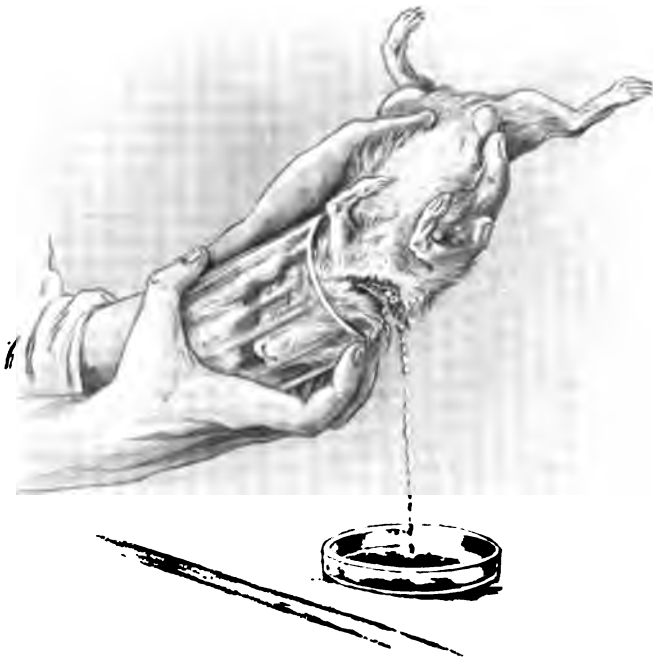


FIG. 9.—Demonstrating method of obtaining complement by bleeding to death an anesthetized guinea-pig.

pig is etherized by placing its head in a glass containing gauze soaked with ether, and before death occurs, the blood-vessels of the neck are severed and the blood collected in a Petri dish (Fig. 9). More blood will be obtained if drawn before respiratory and cardiac movements cease, and from 10 to 15 c.c. should be collected in this manner. The blood is allowed to coagulate, and the clear serum to separate, which it does in about two hours. The serum is then drawn off with a capillary pipette and diluted in the proportion of one part in ten with 0.85 per cent. salt solution, *i.e.*, to 1 part of serum 9 parts of salt solution are added. The complemental property of serum is soon lost, and the guinea-pig serum should therefore be used on the day it is obtained, or not later than the following day, if kept on ice over night.

The different reagents having been prepared as above described, we are now ready for the titration. The rabbit's serum, after having been inactivated, is made up in the following dilutions: 1-500, 1-1000, 1-1500, 1-2000, 1-3000, 1-4000, and 1-5000, in normal salt solution. In a series of test-tubes 1 c.c. of each of these dilutions is placed, together with 1 c.c. of a 5 per cent. suspension of sheep's corpuscles and 0.1 c.c. of the 1-10 dilution of guinea-pig complement, with sufficient salt solution to bring the total volume up to 4 c.c. After their contents have been thoroughly

mixed by inversion, the tubes are placed in the incubator for one hour at 37° C. After this time, if the amboceptor is sufficiently powerful for practical use, the corpuscles in the tubes containing 1-500, 1-1000, 1-1500, and 1-2000 dilutions of rabbit's serum should be completely hæmolyzed. Those in the tubes containing higher dilutions generally show partial hæmolysis. The following table illustrates the titration of hæmolytic amboceptor:

	Amboceptor		Comple- ment	5 per cent. suspen- sion of sheep's cor- puscles	Incubation 1 hr. at 37° C.	Result
	c.c.	dilution	c.c.	c.c.		
1	1	1-500	0.1	1		Complete hæmolysis
2	1	1-1000	0.1	1		Complete hæmolysis
3	1	1-1500	0.1	1		Complete hæmolysis
4	1	1-2000	0.1	1		Complete hæmolysis
5	1	1-3000	0.1	1		Almost complete hæmolysis
6	1	1-4000	0.1	1		Partial hæmolysis
7	1	1-5000	0.1	1		Partial hæmolysis

If the 1-2000 tube does not show complete hæmolysis, the rabbit must be given another injection of sheep's corpuscles in order to raise the hæmolytic power of the serum. If the serum is found to be of sufficient hæmolytic strength, the rabbit should be etherized and bled to death from the carotid artery without delay, as the serum in the living animal will lose its hæmolytic power after a time. The rabbit's blood is collected in Petri dishes, and set aside on ice until the clear serum separates. This is drawn off

with a capillary pipette, inactivated by heating for half an hour at 56° C., and stored for future use in 1 c.c. glass ampoules. Amboceptor, when collected in this way under aseptic conditions, will retain its hæmolytic power for several months. We have found that the serum is less likely to deteriorate or become contaminated when mixed with an equal quantity of glycerin, as suggested by Much. The glycerin in no way interferes with the Wassermann test. We also prefer to store the glycerinized serum in short lengths of glass tubing with sealed ends, as suggested to us by Laird, because a larger amount of air must be enclosed in the ampoules, with consequent greater risk of contamination. In the Wassermann test itself we employ 1 c.c. of a 1-1000 dilution of hæmolytic amboceptor that gives a titre of at least 1-2000. We avoid having to open a fresh tube of amboceptor and thus wasting a great deal, by making a 1-100 dilution from one tube, placing it in a sterile flask, and making the 1-1000 dilution from this for several days.

SYPHILITIC SYSTEM

Antigen.—For reasons given above, we prefer to use as antigen an alcoholic extract made from the liver of a syphilitic foetus that has been found on microscopic examination to be rich in treponemata. The method usually employed in preparing such an anti-

gen is as follows: One part of the tissue is minced and mixed with four parts of 96 per cent. alcohol. The antigenic substances are then extracted by shaking the mixture in a machine for 24 hours, when the fluid is filtered or allowed to stand until the sediment falls to the bottom of the vessel. The clear fluid is then pipetted off and placed in dark bottles, to be used as required. This extract must be tested for antigenic power against a number of known syphilitic and known non-syphilitic sera, and must give positive results with the former and negative with the latter in order to be fit for use. Furthermore, the most suitable dose must be ascertained. When present in excessive amount, the extract may cause fixation of complement alone in the absence of syphilitic serum. The optimal dose of antigen is that which gives complete complement-fixation (or inhibition of hæmolysis) with a syphilitic serum and three times which is required to produce partial fixation without syphilitic serum. Briefly, the optimal dose of antigen should not be greater than one-third the anti-complementary dose. This dose is determined by performing the Wassermann test on a syphilitic serum using several tubes containing the serum and various amounts of antigen. Other series of tubes are set up containing 2, 3, and 4 times these amounts of antigen but without syphilitic serum, to determine the anti-complementary

dose of antigen. This procedure will be understood more clearly after the Wassermann test itself has been described (Fig. 10). In our own work we rely entirely upon an imported German extract made as described above, which we have found to retain its anti-

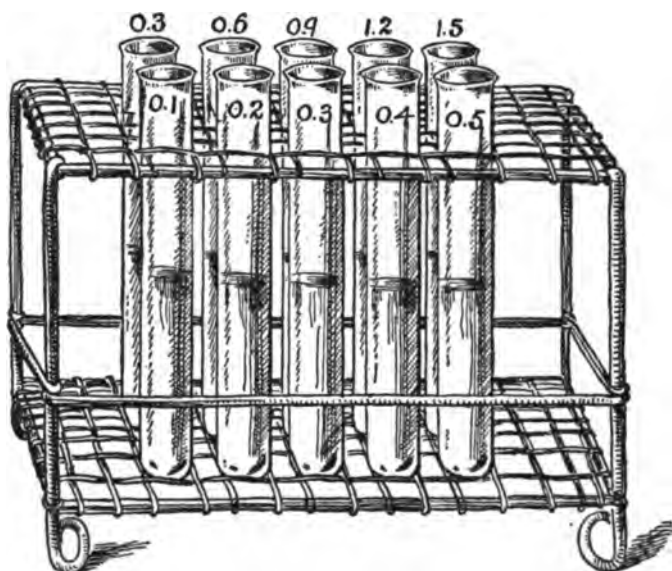


FIG. 10.—Titration of antigen.

Front row: Increasing quantities of antigen tested with 0.1 c.c. of known syphilitic serum to ascertain optimal dose.

Back row: Determination of anti-complementary dose of antigen. Each tube contains 3 times the amount of antigen present in corresponding tube of front row, but no serum.

genic power almost indefinitely, and which has very slight anti-complementary action. By its use we avoid the rather tedious preparation and titration of antigen, except, of course, that we make a test of anti-complementary power with each new bottle, and use

known positive and negative controls every day the test is performed.

Collection and Preparation of the Patient's Serum.

—The patient's blood to be tested may be drawn from a vein at the bend of the elbow with a hypodermic syringe and immediately transferred to a small test-tube. There are several other methods in use, as by means of Keidel's vacuum ampoule (Fig. 11), cup-

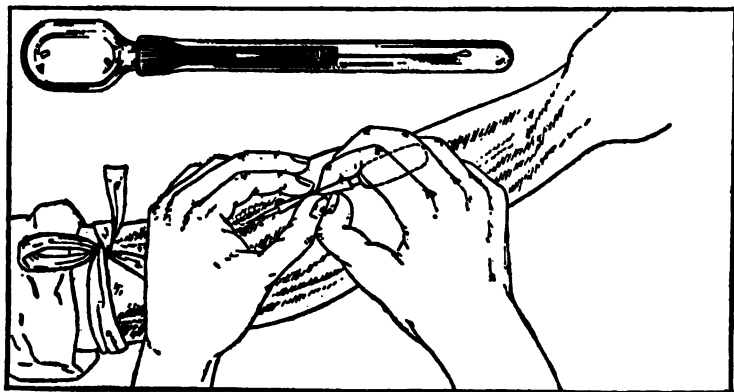


FIG. 11.—Showing method of collecting blood from vein of arm with Keidel's vacuum ampoule. Observe the tourniquet of rubber dam secured with the easily detached, tucked-under loop.

ping, etc. At least 2 c.c. of blood should be obtained. The method employed by us is as follows: The ring-finger is wiped off with alcohol, allowed to dry, and then given a deep puncture at the radial extremity of the distal phalanx about a quarter of an inch below the angle of the nail with a large Hagedorn needle. The blood is then squeezed out into a small test-tube by intermittently compressing the radial side of the

patient's finger with the tip of the little finger of the right hand holding the test-tube, the thumb and index-finger of the left hand holding and constricting the circulation on the other three sides of the patient's finger (see Fig. 12). With a little practice, sufficient

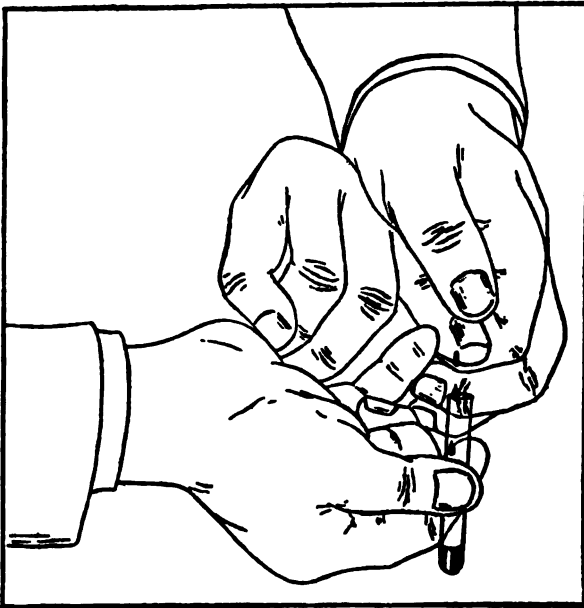


FIG. 12.—Showing authors' method of obtaining blood for complement-fixation reactions. Note the little finger of the right hand holding the test-tube, free to exert intermittent pressure over the radial aspect of the patient's punctured finger, held and constricted on three sides by the enveloping thumb and index-finger of the left hand.

blood can thus be obtained in a minute or two, and the method does not entail any formidable preparation and obviates the possibility of phlebitis incident to faulty venous puncture. In very young children, sufficient blood can quickly be obtained by puncturing

the heel. The tube containing the blood thus collected should be plugged with cotton and if possible be kept in the refrigerator until required for the test. It will keep well for four or five days in this way, but should be tested as early as possible after removal from the patient. If blood is to be carried or sent any distance, a cork or other impervious stopper should be put in the tube, as inversion would cause the serum to be absorbed by a cotton stopper. The blood if kept too long, or in too high temperature, may hæmolyze, and is then unfit for use, though slight hæmolysis does not interfere with the test. Consequently if the serum is to be shipped from a distance or stored unusually long, the serum had best be separated from the clot.

We now arrive at the procedures to be carried out on the day of the test. They will be given, as far as possible, in the order in which they may be carried out in the shortest possible time.

Preparation of Complement.—The guinea-pig is first of all bled to death in the manner previously described, so that its serum will have separated by the time it is required. One guinea-pig as a rule will furnish sufficient complement for about 40 cases and their controls. The serum is made into a 1 in 10 dilution with normal saline solution for convenience in handling small quantities.

Preparation of the Suspension of Sheep's Cor-

puscles.—Sixty c.c. of a 5 per cent. suspension of sheep's corpuscles will usually be found ample for about twenty cases. This suspension is made as described under the hæmolytic system. The washed corpuscles will keep on ice without deterioration for about three days, after which spontaneous hæmolysis usually begins.

Titration of Complement.—In performing the Wassermann test it is essential that we know the smallest amount of complement that is necessary to produce hæmolysis in the hæmolytic system. Therefore before coming to the test proper we must each day, first of all, find out the smallest amount of guinea-pig serum in the presence of which 1 c.c. of a 1-1000 dilution of amboceptor will hæmolyze 1 c.c. of a 5 per cent. suspension of sheep's corpuscles. This amount is known as *1 unit* of complement, and may vary somewhat in the serum of different guinea-pigs. The employment of not more than one unit of complement in the reaction is of great importance, since an excess might furnish enough to produce complete hæmolysis and thus give a negative result when some of the complement employed has been previously fixed by syphilitic serum and antigen. Again, the use of less than one unit of complement would give a positive reaction even in the absence of syphilitic serum, as hæmolysis would then be incomplete. The

majority of workers employ excessive doses of complement and double or treble the smallest amount of amboceptor necessary to produce complete hæmolysis of the corpuscles. The only possible reason for the use of such excessive doses of the two reagents is to overcome the effect of fixation of a slight amount of complement, non-specifically, by the patient's serum and the antigen alone, and to replace the complementary power lost in the incubation. This empirical method is responsible in part for negative results in positive cases. By the method in use by us, we are enabled to accurately allow sufficient complement for the overcoming of any non-specific fixation by serum and antigen alone, and also to make up for the complement lost during incubation, and at the same time use in the test proper not more than one hæmolytic unit. Previous to titration of complement with hæmolytic amboceptor and corpuscles, the complement is first incubated with one dose of antigen and 0.1 c.c. of a mixture of several non-syphilitic sera. This is done for the reason that a slight amount of complement is fixed under these conditions by the serum and antigen, and we are thus sure of allowing for this slight non-specific fixation in the test itself. Different sera present variations in the amount of complement they are capable of absorbing in this

manner, but these variations are usually so slight that for practical purposes they can be ignored, particularly if a pooled serum from several non-syphilitic cases be used. The technic of titration of complement is carried out as follows: Into each of a series of six tubes is placed 0.2, 0.3, 0.4, 0.5, 0.6,

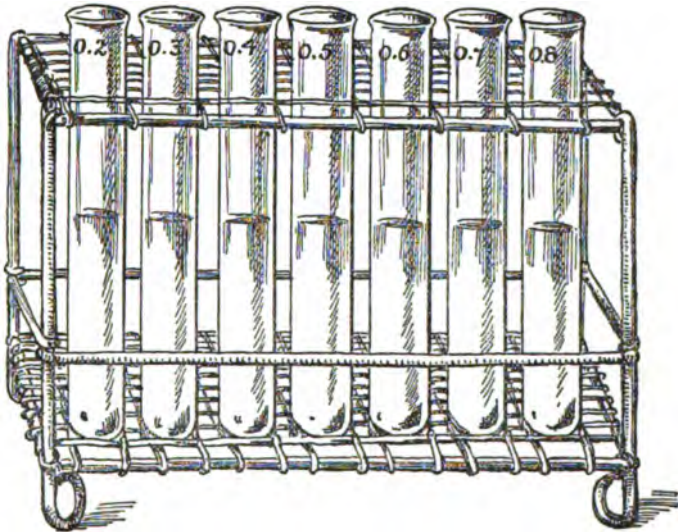


FIG. 13.—Titration of complement. With the complement in each tube are placed 0.1 c.c. of non-syphilitic serum and 1 dose of antigen. The tubes contain gradually increasing amounts of complement, ranging from 0.2 c.c. (dilution 1-10) to 0.8 c.c.

0.7 and 0.8 c.c. of 1-10 dilution of guinea-pig serum, together with 0.1 c.c. of pooled non-syphilitic serum previously inactivated by heating at 56° C. for half an hour, and the dose of antigen used in the test (Fig. 18). Sufficient salt solution is then added to bring the volume up to 2 c.c. The tubes are shaken and incu-

bated for one hour at 37° C., after which 1 c.c. of 1-1000 hæmolytic amboceptor and 1 c.c. of 5 per cent. suspension of sheep's corpuscles are added to each tube. After a further incubation of one hour, the degree of hæmolysis that has taken place in the different tubes is noted. Usually complete hæmolysis has occurred in all the tubes containing 0.5 c.c. and more of diluted complement. The 0.5 c.c. tube is therefore said to contain *one unit* of complement plus the small extra amount allowed for non-specific inhibition due to the presence of patient's serum and antigen. This is the amount of complement to be used in the test proper on that particular day. The table given below will illustrate the titration of complement:

	Pooled normal serum	Antigen 1-7	Comple- ment 1-10	Incubation 1 hr. at 37° C.	Ambo- ceptor 1-1000	Sheep's corpus- cles 5 per cent.	Incubation 1 hr. at 37° C.	Result
1	c.c. 0.1	c.c. 0.2	c.c. 0.2		c.c. 1	c.c. 1		Slight hæmolysis
2	0.1	0.2	0.3		1	1		Partial hæmolysis
3	0.1	0.2	0.4		1	1		Almost complete hæmolysis
4	0.1	0.2	0.5		1	1		Complete hæmolysis
5	0.1	0.2	0.6		1	1		Complete hæmolysis
6	0.1	0.2	0.7		1	1		Complete hæmolysis

By making two parallel experiments, it is readily shown that where the complement has been previously subjected to incubation with inactivated normal serum and antigen, in the same doses as are used in the test itself, a larger amount is required for hæmolysis than

where complement after being incubated for one hour alone is added to the amboceptor and sheep's corpuscles. In a second series of tubes prepared in this way the results would be:

	Complement 1-10	Amboceptor 1-1000	Sheep's corpuscles 5 per cent.	Incubation 1 hr. at 37° C.	Result
	c.c.	c.c.	c.c.		
1	0.2	1	1		Partial hæmolysis
2	0.3	1	1		Almost complete hæmolysis
3	0.4	1	1		Complete hæmolysis
4	0.5	1	1		Complete hæmolysis
5	0.6	1	1		Complete hæmolysis

By this means we arrive at the exact amount of guinea-pig serum that represents one unit of complement. This will be of practical value later as seen in quantitative tests.

Patient's Serum.—The serum of the patient's blood will usually separate spontaneously from the clot and can be drawn off with a capillary pipette. If not, the clot can be broken up well with a platinum loop and the serum separated by centrifuging the tube. The clear serum can then be readily drawn off. The patient's serum is heated in a water-bath at 56° C. for half an hour to destroy the natural complement present (inactivation). This can be carried out while the complement is being titrated. At the same time a known syphilitic and a known non-syphilitic serum should be inactivated to be used in the test as controls.

PERFORMANCE OF THE WASSERMANN TEST PROPER

The performance of the test on one unknown serum with positive and negative controls will be described, though of course any number of sera can be tested simultaneously, using one set of controls.

Six small test-tubes are arranged in two rows, three in each row. Tubes 1 of the front and back rows are for the serum to be tested; Tubes 2 for the known syphilitic serum; and Tubes 3 for the non-syphilitic serum. The back row tubes serve as controls for those of the front row.

Into Tube 1 of the *front* row are placed: 0.1 c.c. of patient's serum; one unit of complement (usually 0.5 c.c. of a 1-10 dilution previously determined by titration); one dose of antigen (usually 0.2 c.c. of a 1-7 dilution).

Into Tube 1 of the *back* row are placed: 0.1 c.c. of patient's serum; one unit of complement; no antigen.

Tubes 2 and 3 are dealt with in the same way, except that 0.1 c.c. of syphilitic serum is used in each of Tubes 2, and 0.1 c.c. of non-syphilitic serum in each of Tubes 3, instead of the unknown serum. The back row tubes furnish controls to show that the serum alone without antigen will not fix complement. The total volume in each tube is now made up to 4 c.c. by the addition of normal salt solution. The contents of the tubes are thoroughly mixed by inversion. The

finger can be used as a stopper for the tube while mixing, provided that contamination from one tube to another is avoided by using a different finger for each case. The tubes are then incubated for one hour at 37° C. to assist in fixation of complement. At the end of this time, no alteration in the appearance of the contents is manifest, though complement-fixation will have taken place in the tubes containing syphilitic serum and antigen. In the negative cases there will be no complement-fixation. The occurrence or non-occurrence of complement-fixation is now determined by the addition of the hæmolytic system and re-incubation of the tubes. To each tube is added 1 c.c. of a 1-1000 dilution of anti-sheep hæmolytic amboceptor and 1 c.c. of a 5 per cent. suspension of sheep's corpuscles. The contents of the tubes are again thoroughly mixed, and the tubes incubated for one and a half to two hours. At the end of this time a preliminary reading of the results may be made, but final reading should be deferred until next morning after the tubes have remained all night in the ice-box.¹

Reading of Results.—In case of a *positive* result, in *Tube 1* of the *front* row there should be no hæmolysis, because complement being fixed by the action of the antigen on the syphilitic serum, no complement re-

¹The time consumed in incubating the tubes may be materially lessened by placing them in a water-bath heated to 37° C. instead of in the air incubator, only one-half the time being required for each incubation in the water-bath.

mains to take part in hæmolysis of the sheep's corpuscles by the amboceptor. The corpuscles will have settled to the bottom of the tube, leaving colorless fluid above. In case of a *negative* result, complete hæmolysis will have occurred in this tube, because there being no "reagin" in the serum to fix complement with the antigen, complement remains free to act with the hæmolytic system during the second incubation. Hæmolysis is shown by a disappearance of the cloudiness due to the corpuscles and the fluid becoming a transparent red color.

In *Tube 2* of the *front* row, containing known syphilitic serum, we should get inhibition of hæmolysis for the same reason that we obtained it in case of a positive result in *Tube 1*.

In *Tube 3* of the *front* row, containing non-syphilitic serum, we should get complete hæmolysis, for the same reason that we obtained it in case of a negative result in *Tube 1*.

In all tubes of the *back* row, there should be complete hæmolysis, because these tubes contain no antigen, and therefore no complement-fixation could have taken place during the first incubation, the complement remaining free to combine with the hæmolytic system. Occasionally, in the serum to be tested, there are substances capable of fixing part or all of the complement in the absence of antigen. In these cases, the

Case to be tested

RESULT

Tube 1

Partial
Hæmolysis Partial
Hæmolysis Complete
Hæmolysis



or



or



Medium + Weakly + Negative
Complete Hæmolysis

Tube 1



Tube 2

:
 j
 j
 a
 a
 y
 r
 a
 k
 j
 r
 t

**S
J
F**

**l
s
r**

pgtinsap

mistake of giving a positive result by absence of hæmolysis in the front row tube is avoided by finding a like result in the control tube in the back row. In reading the results, varying degrees of hæmolysis may be observed in tests of different sera, ranging from complete hæmolysis in negative cases, to absence of hæmolysis in strongly positive cases. Even slight degrees of inhibition of hæmolysis are generally to be regarded as positive, the closeness of reading of borderline cases being dependent on clinical facts and the experience of the worker. The stronger the serum in syphilitic "reagin," the more complement will be fixed, less being left free for hæmolysis. In a serum containing smaller amounts of the Wassermann substance, only part of the complement will be fixed, leaving the remainder free, and we get partial hæmolysis (see Plate II).

Quantitative Estimation.—While the degree of hæmolysis produced gives an idea of the strength of the reaction up to absorption of one unit of complement, yet it does not make any measure of positive cases in which the serum is capable of absorbing more than one unit of complement. In other words, absence of hæmolysis as seen by the usual method means a strongly positive reaction, but indicates no difference in the degree of strength of strongly positive sera; so that a serum may show no signs of weakening under

the effects of treatment when tested with only one unit of complement, because it was previously capable of fixing two or more units. When we wish to determine accurately the complement absorption power of a

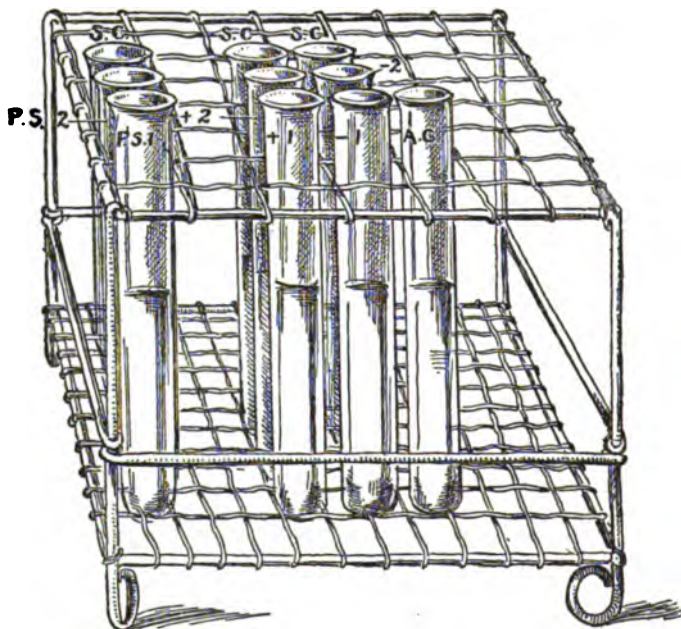


FIG. 14.—Showing arrangement of tubes in performance of Wassermann reaction on one unknown case, with positive and negative controls.

P.S.1. Tube containing patient's serum and 1 unit of complement. P.S.2. Tube containing patient's serum and 2 units of complement. S.C. Serum control tubes, without antigen. +1, +2. Tubes containing known syphilitic serum and 1 and 2 units of complement respectively. -1, -2. Tubes containing known non-syphilitic serum and 1 and 2 units of complement respectively. A.C. Antigen control, containing a double dose of antigen.

serum, in order to gauge the effects of treatment by subsequent tests, tubes should be prepared in doing the test containing two, three, or more units of complement in addition to the usual one unit (Fig. 14).

By this means we can readily see whether the usual amount of the serum used is capable of fixing one, two, three, or more units of complement. It is rarely necessary to carry this quantitative determination beyond two units of complement, though some sera will fix five or six units. By means of a series of tests at intervals on a case under treatment, we can observe that the serum gradually loses its power to fix complement, absorbing perhaps two units the first time, then one unit, then allowing hæmolysis of half the corpuscles, then only a few corpuscles being left unhæmolyzed, and finally complete hæmolysis showing that the serum has become negative. Some workers use signs to designate the strength of the Wassermann reaction, a weak reaction being indicated by + or 1 plus, meaning that there was only 25 to 50 per cent. inhibition of hæmolysis, between 50 and 75 per cent. inhibition being indicated by ++ or 2 plus, between 75 and 100 per cent. inhibition as +++ or 3 plus, and complete inhibition as ++++ or 4 plus. The percentage of hæmolysis cannot be thus accurately guessed at by the eye, and moreover this method of notation does not give the strength of the reaction beyond specific absorption of one hæmolytic dose of complement. We prefer to designate reactions up to one unit of fixation as *negative*, *weakly positive*, *medium positive*, and *strongly positive*, giving the number of units of complement ab-

sorbed in reactions stronger than this. We believe that this method of designation conveys more information as a rule to the clinician than the plus signs. We are indebted to Dr. John L. Laird for the principles we follow in quantitative determination (*Pa. Med. Jour.*, 1911-12, xv, 97-102). For those who wish to measure the percentage of hæmolysis more exactly, the Duboscq colorimeter may be used according to a method described by one of us (Ivy, *Jour. Amer. Med. Assn.*, Aug. 10, 1912, p. 432). We regard this accurate measurement as seldom called for, yet the method may be found useful to those who are in the habit of guessing the percentage of hæmolysis in giving their results.

The sera that have been tested can be preserved and used as positive and negative controls in future reactions, provided they are reactivated by heating before subsequent tests are made.

The table (Plate II) illustrates the performance of the test on an unknown serum, with positive and negative controls.

MODIFICATIONS OF THE WASSERMANN REACTION

Each worker is apt to vary from the original technic in minor details to suit his own convenience in the performance of the Wassermann reaction. These slight modifications are in no way to be regarded as radical changes in the reaction. By the original Was-

sermann reaction, as we view the matter, is meant the use of extract of syphilitic liver as antigen, an anti-sheep hæmolytic system, fresh guinea-pig serum as complement, and inactivation of the patient's serum. Various workers have sought to improve on the original Wassermann technic by the use of other antigens, as above mentioned; by using anti-ox, anti-pig, anti-human, and other hæmolytic systems; by using fresh instead of inactivated patient's serum, etc. All attempts to simplify the technic have resulted in sacrificing the delicacy of the test. The modification that is most commonly employed in this country is that of Noguchi, who, realizing the value of the laboratory reaction for syphilis to the medical profession and the training and experience necessary for the performance of the test as laid down by Wassermann, attempted to simplify the technic so as to make it accessible to the general practitioner as a routine office procedure. He proposed the use of an anti-human hæmolytic system; thus making unnecessary the use of sheep's blood. He prepared anti-human hæmolytic amboceptor by injecting rabbits with human corpuscles. The antigen, amboceptor, and complement were put up in dry form by saturating strips of absorbent paper with these reagents. In the performance of the test, it was only necessary to add the antigen and complement papers to diluted patient's serum, incubate, and then add the

amboceptor paper and washed corpuscles from the patient. But it was soon found that the complement paper rapidly lost its power and became useless, leading Noguchi to discard it in favor of fresh guinea-pig serum. The antigen and amboceptor papers are still employed to some extent, but accurate titrations of the reagents cannot be carried out when they are used, and misleading results are liable to occur. Reliable workers employing the Noguchi system now use the different reagents in their original fluid form, titrating them accurately, so that the technic as carried out at present has no advantage over the original Wassermann in point of simplicity. Those accustomed to the Noguchi modification with careful titration of all the reagents, no doubt obtain as reliable results as can be obtained with the Wassermann technic, and even claim that the results are more delicate. We believe, however, that by proper titration and employment of a minimal amount of complement, there will be little difference in the results obtained by the two methods. As in all laboratory procedures, cases will be encountered on the border-line between positive and negative which can only be judged by a consideration of the clinical features of the cases, and the experience of the worker.

Hecht-Weinberg Modification.—The Hecht-Weinberg test modifies the Wassermann reaction for

syphilis by utilizing the natural complement and anti-sheep hæmolyisin present in the patient's serum instead of adding guinea-pig serum and prepared hæmolytic amboceptor. Gradwohl (*Jour. Amer. Med. Assn.*, July 18, 1914) regards this test as of great value as a check upon the Wassermann reaction. In a series of one thousand cases he found the Hecht-Weinberg was positive 15 per cent. more often than the Wassermann.

CLINICAL APPLICATION OF THE WASSERMANN REACTION

In a great many cases, the correct interpretation of the examination of the patient's serum in the laboratory can only be given when the clinical aspect of the case is considered. The clinician therefore should be familiar with the meaning of a given result from the laboratory so that he can intelligently apply it to the case in question. For instance, many practitioners are disappointed upon the receipt of a negative Wassermann report in a case clinically diagnosed as syphilis, because the blood has been collected from the patient when under the influence of specific treatment. A familiarity with the following facts will be of value in interpreting the results of the reaction.

The question as to whether the Wassermann reaction is specific for syphilitic infection alone or whether it is found in other diseases, has been the occasion for

much study. Observers from time to time have obtained positive results in yaws or frambœsia, trypanosomiasis, some cases of leprosy, malarial fever, scarlet fever, and occasionally in other diseases. It is not surprising that a positive result should be given in yaws, a disease clinically similar to syphilis in many respects, and which is due to the *Spirochæta pertenuis*, an organism very difficult to distinguish from the *Treponema pallidum*. Positive results with the Wassermann reaction are found more frequently in cases of the tuberculous type of leprosy than the anæsthetic form. In malaria the reports are conflicting. Some observers have obtained positive results in a certain percentage of cases, which become negative as soon as the malaria parasites disappear from the blood. Others have had uniformly negative results. Similar experiences have been reported in scarlet fever. After summing up all the data at our disposal, and judging from our own results, we must conclude that except in yaws, trypanosomiasis, and leprosy, a *positive* Wassermann reaction means that the patient is the victim of syphilitic infection. The diseases named being practically unknown in temperate climates, should cause no confusion in this part of the world. The positive results obtained in other diseases are either due to the fact that syphilis cannot be absolutely excluded, or to faulty technic.

On the other hand, we may obtain a *negative* Wassermann reaction in the presence of syphilitic infection under certain conditions to be mentioned below in detail.

Primary Stage of Syphilis.—The Wassermann reaction as a rule does not become positive until at least two weeks after the appearance of the chancre, the average time being from three to four weeks, though cases have been known in which it was positive before the chancre appeared. In our records the earliest positive Wassermann reaction was obtained four days after the appearance of the primary lesion. Occasionally it does not become positive until after the secondary symptoms have manifested themselves. The Wassermann reaction has proved that many instances of so-called “soft sores” are in reality due to syphilitic infection, or at least a mixed infection exists. The earliest means of making an absolute diagnosis of the primary lesion of syphilis is by finding the *Treponema pallidum* in the secretion from the sore by dark field illumination or by stained smears. This method fails, however, in a certain percentage of cases, especially when local treatment has been applied; so that, while a negative Wassermann reaction in the suspected primary stage of syphilis does not exclude the disease, a positive reaction will frequently serve to establish the diagnosis.

Secondary Stage.—The reaction is positive in practically all cases of secondary syphilis, and is therefore of the greatest value in making the diagnosis of atypical cases. In this stage, too, we usually find the strongest reactions.

Tertiary Stage.—In tertiary syphilis a positive Wassermann reaction is obtained in 80 to 90 per cent. of cases. A negative reaction in this stage therefore does not have quite the same significance as in suspected secondary syphilis. Medium and weakly positive reactions are more frequent here than in the secondary stage.

Latent Syphilis.—By latent stages are meant periods in which all symptoms disappear for a time, either spontaneously or as a result of treatment, but in which, as shown by the Wassermann test, the specific reaction between the spirochætæ and the tissues is still going on, indicating therefore that the disease is not eradicated. It has been found that persons in this stage, although exhibiting no symptoms, yet are capable of infecting others, and themselves are liable to develop later manifestations of the disease. It is estimated that about 50 per cent. of latent syphilitics give a positive Wassermann reaction. It is difficult to estimate this percentage, because we cannot always say whether a given case having a negative Wassermann reaction is cured or merely in the latent stage.

Craig and Nichols (*Jour. Amer. Med. Assn.*, 1911, lvii, p. 474) have found that the recent ingestion of varying quantities of alcohol has a marked effect on the Wassermann reaction. These authors state that from 180 to 240 c.c. of whiskey, 90 c.c. of 95 per cent. alcohol, and 700 c.c. of Munich beer are capable of causing a positive reaction to become negative for periods ranging from 24 to 72 hours. They therefore recommend, in any case where the ingestion of large quantities of alcohol in the preceding 24 hours is suspected, that the removal of blood from the patient for performance of the test be postponed for 3 or 4 days. The authors have recently observed a case of secondary syphilis in a chronic alcoholic patient, whose Wassermann did not become positive until the seventh week of the disease. Previously, in an extensive experience with hundreds of cases of untreated syphilis, the Wassermann reaction always resulted positively by the fifth week following the appearance of the chancre.

It is now pretty generally agreed that a positive Wassermann reaction means the presence of living spirochætæ in the body, the organisms having been found in practically all lesions of every stage of acquired and inherited syphilis. The reaction differs from true antigen-antibody reactions in that the latter are apt to persist for some time after the infecting

agent has disappeared from the body, while the Wassermann "reagin" usually disappears very rapidly.

In *inherited syphilis* the Wassermann reaction is positive in a very high percentage of cases, at least 90 per cent. The reaction tends to show moreover that a great many congenital defects and maladies of young infants, until recently regarded as due to syphilis, are not caused by this disease. The Wassermann reaction has thrown considerable light on the significance of the laws of Colles and Profeta. *Colles' law* is that the mother of a syphilitic child may show no signs of syphilis and is immune to the disease. It has been found that in a very high percentage of such mothers the blood gives a positive reaction. These cases probably have syphilis in a modified form, and therefore cannot be reinfected. *Profeta's law* is that the children of syphilitic mothers may be apparently healthy, and yet not susceptible to syphilitic infection. A high percentage of these children also give a positive Wassermann reaction, the explanation being the same as in the case of Colles' law.

Parasyphilitic Affections.—Under this name are grouped certain diseases of the nervous system, particularly paresis and tabes dorsalis. The discovery of the *Treponema pallidum* in the lesions of paresis and tabes has proved them to be true manifestations of syphilis in the vast majority of cases. The Wasser-

mann reaction may be performed on cases of these diseases by examination of either the blood-serum or the cerebrospinal fluid. In using the latter for the test inactivation is not necessary, as the fluid contains no complement. The technic is the same as when using blood-serum, except that 0.2 c.c. of the cerebrospinal fluid should be employed. According to most observers, in *paresis*, examination of the blood-serum shows the Wassermann reaction to be positive in practically all cases, while the cerebrospinal fluid gives a positive result in about 90 per cent. of cases.

In *tabes* both the blood-serum and the cerebrospinal fluid give a positive Wassermann reaction in 60 to 70 per cent. of cases.

In the manifestations of syphilis of the central nervous system, which do not differ materially from syphilitic lesions elsewhere, and are grouped apart from paresis and tabes, the Wassermann reaction with the blood-serum is positive in practically all cases, while it is positive in only a small percentage of cases with the cerebrospinal fluid. In this group are included gummatous lesions, syphilitic meningitis, etc. From personal experience, we have found in general that the cerebrospinal fluid is more frequently positive or gives a stronger Wassermann reaction than the blood-serum in the majority of cases of syphilis involving the central nervous system.

The Wassermann reaction has shown that a high percentage of cases of aortic disease and aneurism are syphilitic in origin.

Effects of Treatment on the Wassermann Reaction.—The Wassermann reaction is markedly influenced by treatment. In a syphilitic undergoing treatment with mercury and particularly salvarsan, the reaction may rapidly be reduced from strongly positive to negative. As a guide or measure of the effects of treatment, therefore, the Wassermann reaction plays almost as important a rôle as it does in diagnosis. True biological antibodies, such as for instance occur in typhoid fever as determined by the agglutination reaction, may persist in the blood for months and even years after the disease has been eradicated. On the other hand, the evidence of the Wassermann reaction shows that the substances taking part in it disappear from the patient's body very shortly after the destruction of the syphilitic virus by treatment, or when the virulence of any remaining spirochætæ is much diminished.

In the primary stage, if treatment by salvarsan or neosalvarsan be instituted as soon as the diagnosis is made (by dark field illumination), the Wassermann reaction may never become positive and secondary symptoms may never appear. With every day that treatment is delayed, and especially after the Wasser-

mann reaction has become positive, a proportionate increase in treatment will be required to eradicate the disease. In the tertiary stage it is impossible in some cases to render the reaction negative.

Under treatment the symptoms usually disappear before the Wassermann reaction becomes negative, though occasionally the opposite holds true; that is, cases under treatment may present a negative reaction although symptoms are still manifest. The reaction may persist for some time after all symptoms have disappeared, indicating that further treatment is necessary. After being rendered negative by treatment, the reaction may after a time again become weakly positive, then gradually stronger, and finally be followed by a reappearance of symptoms. It is not yet possible to say when a negative reaction in a case that has received treatment means that the patient is cured of syphilis. If negative Wassermans have been obtained at intervals of three or four months over a period of two years, during which the patient has had no treatment and with no recurrence of symptoms, it is probably justifiable to regard the case as cured. But it will require ten to twenty years' experience with the reaction to be positive that the so-called parasyphilitic diseases or other late manifestations will not develop even after such a series of negative tests.

Much closer readings of the reaction should be

made in using it as a guide to treatment than as a diagnostic aid. Border-line reactions giving very slight inhibition of hæmolysis (5–10 per cent.) when performed for diagnosis should be regarded as negative. But such a result in a case that has been known to have had syphilis and to have been under treatment indicates that further treatment is required.

Occasionally in a case of late or treated syphilis giving a negative Wassermann reaction, a positive result may be obtained immediately after a so-called *provocative dose* of salvarsan, neosalvarsan or mercury. It is believed that the treatment in such cases is just sufficient to stimulate the spirochætæ without causing their death.

In view of the fact that treatment by mercury or salvarsan often produces a negative reaction in cases where it otherwise would be positive, the test should not be performed on a patient unless all treatment has been discontinued for at least three weeks. If this precaution be not observed, negative results will frequently be obtained in cases that should be positive, greatly to the surprise and chagrin of the practitioner.

In employing the Wassermann reaction in differential diagnosis, it must be remembered that a positive result does not necessarily mean that the lesion in question is syphilitic, but that there is syphilitic infection in the body of the patient.

XIII

FIXATION OF COMPLEMENT (Continued)

GONOCOCCUS COMPLEMENT-FIXATION TEST—SERUM DIAGNOSIS OF ECHINOCOCCUS DISEASE—COMPLEMENT-FIXATION REACTION IN TYPHOID FEVER—COMPLEMENT-FIXATION REACTION IN TUBERCULOSIS—THE COMPLEMENT-FIXATION REACTION AS APPLIED IN PROTEIN DIFFERENTIATION (NEISSER-SACHS REACTION)

THE GONOCOCCUS COMPLEMENT-FIXATION TEST

IN recognition of the recent admirable work by Schwartz and McNeil on the complement-fixation test in gonococcic infections, the fact must not be overlooked that Müller and Oppenheim, in 1906, were the first to apply this reaction to a gonorrhœal affection and consequently are entitled to the distinction of being termed the originators. The present popularity of this test has been the outgrowth of the suggestion made by Schwartz and McNeil—namely, that of the employment of a polyvalent antigen. As a result of their labors, these workers contend, and seem to have proved conclusively, through animal experimentation: (1) “that the different strains of the gonococcus differ markedly one from another—so much so that the antibodies produced in the body by the toxin of one strain will in many instances not bind the complement in the presence of an antigen prepared from another strain. Therefore, if only one strain is used in the preparation of the antigen, a great many nega-

tive results would be obtained in positive cases; (2) an antigen prepared from many strains fixes the complement whenever one of its component strains does so, and consequently the necessity of testing a serum against a number of antigens separately is avoided. It is not to be denied that there probably are other strains of gonococci differing widely from any present in the polyvalent antigen, so that at times a negative result will be obtained in a positive case."

While we recognize the fact that a negative reaction may mean nothing, in fact, may be erroneously contradictory, the significance, on the other hand, of a positive reaction has been so great—more specific, in fact, than when the lipotropic antigen, commonly employed in the performance of the Wassermann reaction, is utilized—that we have applied the test in a large series of diverse cases with the most gratifying results.

Discussion of Technic.—Schwartz's method and the technic which we practiced for a time in comparison with our own gonococcus complement-fixation test is as follows, so far as the quantities of the ingredients participating in the reaction are concerned: (1) patient's serum, 0.02 c.c.; (2) salt solution, sufficient to equalize the volume in each tube; (3) antigen (routinely 0.3 c.c. in one tube and 0.15 c.c. in another tube, of a commercial preparation diluted 1 to 10). These quantities are determined by preliminary standardiza-

tion with a fresh known negative and a known positive serum; the positive showing the true antigenic dose, and the negative the highest quantity of antigen which will allow complete hæmolysis; in the actual test this large quantity of antigen is placed in one tube and one-half the quantity in a second tube; (4) complement 0.1 c.c. of a dilution 1 to 10; (5) amboceptor, 0.1 c.c. representing twice the lowest quantity that will completely hæmolyze 0.1 c.c. of the cell suspension with 0.1 c.c. of complement in one hour; (6) sheep's red blood-cells (5 per cent. suspension), 0.1 c.c. Incubation is made for one-half hour at 37° C. in a water-bath or for one hour in dry heat, before and after the addition of the hæmolytic system.

The technic on which we have learned to place the greatest reliance is essentially that utilized by us in the performance of the Wassermann reaction, merely substituting the gonococcus specific antigen for the syphilitic lipotropic antigen, employing always the carefully standardized single complement unit and the routine standardization of antigen and amboceptor (see Table of Test Reactions).

Antigens.—The necessity of a polyvalent antigen is indisputable, presumably owing to the diversity of the strains of the gonococcus. The only question of importance is, how may this antigen be prepared to the best advantage? Schwartz and McNeil in their latest communication say that the "various strains of

gonococci are grown on salt-free veal-agar, neutral in reaction to phenolphthalein: 24-hour-old cultures are washed off the agar-slants with distilled water and the resulting suspension is heated for two hours in a water-bath at 56° C. It is then centrifuged and passed through a Berkefeld filter. No salt is added to this antigen until it is desired to use it, when it is made up to 0.9 per cent. strength by adding one part of 9 per cent. saline solution to nine parts of antigen. Following Schwartz and McNeil's instructions to the letter, we have prepared monovalent, trivalent and hexavalent antigens and have employed them comparatively in a large series of cases with the result that the hexavalent preparation gave the highest percentage of positive results and in every way appeared to be the most reliable of the three antigens. Even with the hexavalent antigen, prepared as described above, we have been forced, in comparative studies, to the conclusion that it has not been so useful or reliable as when prepared in the following manner: Forty-eight-hour-old cultures of the same six strains of gonococci, grown on blood-agar, were washed off in sterile distilled water; shaken for one hour; and autolyzed for twenty-four hours in a thermostat at the temperature of 37° C. and heated in a water-bath at 60° C. for one-half hour. Before use, this antigen is diluted one to ten by the addition of 0.85 per cent. salt solution.

Analysis of Cases Treated.—The result of our work has been little more than a corroboration of the reports that have emanated from Schwartz and McNeil and those who have confirmed their results. We believe, however, that, by utilizing the technic herein described, we have added to the accuracy of the test as applied by them and have thereby improved the findings to the credit of the test and its value in clinical diagnosis. To the increased positive results we attribute the accurate standardization of antigen on each occasion and the employment of a standardized single complement unit.

Reviewing our experience with the gonococcus complement-fixation test in general, it may be stated that a negative reaction is not decisive against the presence of a gonorrhoeal infection, and this is particularly true during the first six weeks of a primary acute urethritis either anterior or posterior in the absence of any complication, previous to which time we have never obtained a positive reaction; on the other hand, the supervention, even during the acute stage of the disease, of complications such as epididymitis, arthritis, prostatitis, etc., is prone to result in the production of a positive reaction. On the contrary in our experience, a positive reaction has been pathognomonic of a focus of gonococcal infection and has assisted many times in elucidating obscure or

doubtful lesions. In fact, it appears that the gonococcus-fixation test enjoys greater specificity than does the Wassermann reaction, since thus far we have found no alien infection or condition capable of producing a positive reaction. This much certainly cannot be claimed for the Wassermann reaction. Moreover, there is no drug, as there is in syphilis, which is capable of causing the reaction to be negative during the existence of the disease. The probable explanation for the greater specificity of the gonococcus complement-fixation test rests in the fact that with gonococcic infections we employ a specific antigen—the gonococcus—while in the case of syphilis a non-specific or lipotropic antigen is employed.

The analysis of our cases further illustrates another interesting feature, namely, the persistence in some cases for a short time of a positive reaction, after an apparent clinical cure. This has occurred so often that we no longer discharge a patient cured or give him a clean bill of health so long as he gives a positive reaction, provided he has not been the recipient of immunotherapy. Usually a persistent positive reaction will become negative in two or three weeks following clinical cure with or without a continuation of treatment. The only explanation is that it requires an indefinite time for the antibodies, formed during the course of infection, to disappear from the blood.

Torrey, in animal experimentation, has found that the antibodies in immunized rabbits begin to disappear after ten days, and that the elimination is practically complete by the fiftieth day in all cases, disappearing much earlier in many instances. Thus a patient, evidencing a positive reaction two months after presumed clinical cure, should be regarded as still harboring a latent gonorrhœal focus. Such experience, adopted either as routine procedure in the management of treatment, or discovered accidentally when gonorrhœal infection or its symptoms were denied, or demonstrated by submitting suspected or positive syphilitic serum to the gonococcus-fixation test, has been encountered in a large number of cases.

Because of the generally acknowledged difficulties, at times, of differentiating the pelvic lesions in women, notably certain of the inflammatory from the cystic and neoplastic conditions, and also the differential diagnosis among gonorrhœal, tuberculous and pyogenic infections themselves, the gonorrhœal-fixation test seems destined to play a rôle. As in the male, in whom a positive reaction seems never to occur, at least so long as the infection is confined to the anterior urethra, so in the female we have been unable to obtain a positive reaction unless the disease has ascended to the level of the uterus.

An interesting, if not important, feature connected

with this work is the comparative importance and value of the serological and bacteriological examination of cases of suspected gonorrhœal infection. It is, to-day, a fact that the judiciary courts of our land require that the presence of the gonococcus be demonstrated, culturally, in order to establish its indisputable and legal identity. Based on this qualification, there are many cases of gonococcic infection impossible to determine, and we do not hesitate to state that, in our judgment, many such cultures are in reality the *Micrococcus catarrhalis* and not the gonococcus. This applies particularly to such isolation of the diplococcus of Neisser from chronic inflammatory processes. Moreover, it must be generally recognized that the demonstration of a Gram-negative diplococcus in smear is often insufficient and faulty evidence on which to base a diagnosis of gonococci. Therefore, it is most fortunate that in the chronic stage of the disease with complications, the complement-fixation test seems to be signally meritorious, while in the acute, subacute and frequently in the chronic forms of the diseases when the gonococcus may be demonstrated bacteriologically, the serological test promises little or nothing.

Conclusions.—Detailed and careful analysis of the gonococcus complement-fixation test, performed with

the serums of the cases tabulated in our series,¹ would seem to justify the following assertions:

1. A positive reaction is invariably reliable and always denotes the presence of a focus of gonococcic infection.

2. A negative reaction frequently occurs in the presence of disease, especially in the acute and sub-acute stages when the disease is limited to the urethra, and it is always negative when the disease is confined to the anterior urethra or vagina alone.

3. In no alien non-gonorrhœal infections of systemic disease has a positive reaction been obtained; the test, therefore, appears to be absolutely specific.

4. A positive reaction has been found to be present in 21.05 per cent. of patients clinically cured. Such patients, therefore, should not be discharged from treatment or observation until a negative reaction has been obtained.

5. Not infrequently, either when suspicious lesions are presented or accidentally, positive reactions will be discovered in patients denying gonorrhœa.

6. In only 9.09 per cent. of cases of acute and sub-acute antero-posterior urethritis has the complement-fixation test resulted positively. The earliest appearance of a positive reaction in a primary attack of

¹ *Archiv. Int. Med.*, January, 1914, p. 143.

posterior urethritis, without complication, occurred in the sixth week.

7. In a number of cases of chronic recurrent urethritis with acute exacerbations, the test was invariably positive; many of these patients undoubtedly had prostatitis.

8. The reaction resulted positively in one-third of all cases of chronic posterior urethritis; undoubtedly many had a mild or low-grade prostatitis.

9. In 52.08 per cent. of cases of chronic prostatitis a positive reaction was obtainable.

10. Two-thirds of all stricture cases demonstrated a positive test.

11. In epididymitis a positive complement-fixation test was observed in 87.5 per cent. of cases. If, from our series, one case, probably tuberculous, may be eliminated, and a time duration of five weeks can be imposed, the positive result in this form of disease has been 100 per cent.

12. In arthritis, undoubtedly gonorrhœal in character, positive reactions were obtained in 100 per cent. of cases.

13. In the diagnosis and differential diagnosis of pelvic disease in women, the gonococcus-fixation test is destined, unquestionably, to play an important rôle. We have been unable to obtain any positive results in uncomplicated urethritis, vulvovaginitis and Bar-

tholinitis, and it would appear that the infection must ascend at least to the level of the uterus in order to produce a positive blood response.

14. Inoculations of gonococcus bacterin, antigonococcic serum, etc., may in themselves by the production of immune bodies be causes of positive reactions. How long these immunizing effects may endure is unknown, but we have observed patients, treated by immunotherapy, who one year later demonstrated negative complement-fixation reactions.

15. Although the bacteriological demonstration of the gonococcus culturally is the only absolute method for its identification in chronic inflammatory processes, the method as a routine procedure is impractical and susceptible of many failures and fallacious results, so that the complement-fixation test is not only less laborious, but is productive of a higher percentage of positive findings.

A series of comparative studies using non-specific with the specific antigens in the performance of the gonococcus complement-fixation reaction has been carried out (Thomas, B. A., Ivy, R. H., and Birdsall, J. C., *Surgery, Gynecology, and Obstetrics*, 1914). Polyvalent antigens were prepared from various non-gonorrhœal Gram-negative and positive bacteria, namely, the *Micrococcus catarrhalis*, the *Diplococcus meningitidis*, the *Pneumococcus*, the *Strepto-*

coccus pyogenes, the *Micrococcus albus* and *aureus*, the *Colon bacillus*, and the *Corynebacterium pseudodiphtheriæ*.

From these later studies we have deduced the following facts: (1) In no case have polyvalent antigens prepared from meningococci, pneumococci, streptococci, staphylococci, colon bacilli, or corynebacteria sufficed to fix complement, thereby not jeopardizing the specificity of the gonococcus antigen. (2) In ten per cent. of sera examined a weakly positive result was obtained with polyvalent *Micrococcus catarrhalis* antigen; in these cases the reaction was much more marked with the various gonococcic antigens. Thus it may be inferred that the relation between the gonococcus and the *Micrococcus catarrhalis* is not positively and absolutely defined and it is not unlikely, on the one hand, that a culture of the *M. catarrhalis* is occasionally included in a supposedly specific polyvalent gonococcus antigen, while, on the other hand, it is undoubtedly true that a mixed infection often due to the *M. catarrhalis* exists in patients suffering from gonorrhœa and its complications.

SERUM DIAGNOSIS OF ECHINOCOCCUS DISEASE

Echinococcus disease is rare in this country in the human being, and therefore serum diagnosis is seldom called for. Results with the complement-fixation test,

however, have shown it to be a reliable method of diagnosis. The technic of the reaction is the same as that for the Wassermann reaction. The antigen used consists of the fluid from hydatid cysts of sheep affected with the disease. Suitable amounts of this fluid when brought in contact with the blood-serum of patients suffering from echinococcus disease will cause complement fixation.

COMPLEMENT-FIXATION REACTION IN TYPHOID FEVER

In reporting the results of the complement-fixation reaction in typhoid fever, Garbat (*Am. Jr. Med. Sc.*, July, 1914) finds that the serum of practically all typhoid fever patients sooner or later gives a positive complement-fixation reaction. A highly polyvalent antigen properly prepared is absolutely essential in order to obtain a maximum of positive results. A positive complement-fixation test throws great corroborative diagnostic weight on the side of a doubtful or positive Widal reaction. Occasionally the test is positive before the Widal or blood culture, but usually not before the end of the second week. It generally persists for about six weeks after recovery.

COMPLEMENT-FIXATION REACTION IN TUBERCULOSIS

Employing as antigen a simple emulsion of living tubercle bacilli, McIntosh, Fildes, and Radcliffe (*Lancet*, Aug. 22, 1914) obtained positive comple-

ment-fixation in 70 per cent. of 85 pathologically certain cases and in 66 per cent. of clinically certain cases of tuberculosis. In 87 controls, taken from normal and disease conditions, without any selection, all were negative but three, these being two cases of leprosy and one of Addison's disease. From these results the reaction may be regarded as highly specific.

THE COMPLEMENT-FIXATION REACTION AS APPLIED TO
PROTEIN DIFFERENTIATION (NEISSER-SACHS
REACTION)

This method may be applied to supplement the results of the precipitation reaction, and may serve to differentiate proteins where the precipitation reaction fails. It is of practical importance in the medico-legal identification of human blood. An anti-sheep hæmolytic system is prepared in the same manner as for the Wassermann reaction. For the identification of human blood, an anti-human serum is made by injecting a rabbit with human blood-serum. The anti-serum should be prepared of such a strength that 0.03 or 0.04 c.c. will give complement fixation with 0.00001 c.c. of human serum. The antiserum having been previously prepared, an extract of the suspected blood is made in approximately 1-1000 dilution in the same manner as for the precipitation reaction (see Chapter X).

The test is carried out according to the following

table, by mixing different quantities of the extract of the suspected blood with 0.1 c.c. of suspected blood and 0.03 c.c. of the antiserum.

Extract of blood		Complement	Antiserum
0.1	c.c.	0.1 c.c.	0.03 c.c.
0.05	c.c.	0.1 c.c.	0.03 c.c.
0.02	c.c.	0.1 c.c.	0.03 c.c.
0.01	c.c.	0.1 c.c.	0.03 c.c.
0.005	c.c.	0.1 c.c.	0.03 c.c.
0.002	c.c.	0.1 c.c.	0.03 c.c.
0.001	c.c.	0.1 c.c.	0.03 c.c.
0.0001	c.c.	0.1 c.c.	0.03 c.c.

The tubes containing the above are placed in the incubator at 37° C. for one hour. Then the doses of hæmolytic amboceptor and 5 per cent. sheep's corpuscles are added and the tubes again incubated for one hour. If 0.01 c.c. of the extract of blood prevents hæmolysis, the test can be regarded as positive for human blood.

Antisera for blood of various animals can be prepared in the same way and the extract of unknown blood tested with them.

XIV

MISCELLANEOUS BIOCHEMICAL REACTIONS

ABDERHALDEN'S BIOLOGICAL TEST FOR PREGNANCY—
SERO-ENZYME TEST FOR SYPHILIS—ABDERHALDEN-
FAUSER REACTION IN MENTAL DISEASES—MEIOSTAG-
MIN REACTION—EPIPHANIN REACTION

ABDERHALDEN'S BIOLOGICAL TEST FOR PREGNANCY

THIS biological test belongs to a class of newer serum reactions. It has been found by Abderhalden of Halle that foreign proteins entering the blood produce specific protein-splitting enzymes. In pregnancy, Abderhalden assumes that the placenta gives off a protein which causes the production of a specific enzyme in the blood which is capable of splitting up the placental protein with the formation of peptone and amino-acids. The test consists therefore of incubating the serum of a suspected pregnant woman with placental material, and then testing the fluid for the end products of protein digestion. Two methods are employed in the detection of these substances, first the *dialysis method*, in which the presence of dialyzable peptones are tested for at the end of the incubation by means of certain color reactions; and second the *optical method*, in which the end products of protein digestion are detected by means of the polariscope.

A brief outline of the technic of the dialysis

method is as follows: Fresh placenta is cut in small pieces and repeatedly boiled with a little acetic acid until all soluble protein is removed. This is determined by testing with the biuret reaction. The patient's serum is collected by puncturing a vein at the elbow. Five or six c.c. of serum should be obtained, and must be absolutely free from hæmoglobin. For dialysis, Schleicher and Schull's diffusion shells are employed. These membranes must be impermeable to the protein of blood-serum and permeable to peptone, as ascertained by preliminary tests. The ninhydrin reaction is used for reading the results. This substance, the full name of which is triketohydrindenhydrat, gives a blue color with the end products of protein digestion.

Into one of the diffusion shells are placed 1 gramme of the boiled placental material and 1.5 to 2 c.c. of patient's serum. The membrane is then placed in a small beaker containing 20 c.c. of water. The fluid in and outside the membrane is then covered with toluol and the whole incubated at 37° C. for 16 to 24 hours. At the end of this time, the water in the beaker is tested for products of proteid digestion by adding to it 0.2 c.c. of a 1 per cent. watery solution of ninhydrin. If the reaction is positive a blue color results. The reaction must be carefully controlled by using at the same time other membranes containing

serum alone and placenta alone. Great care must also be exercised to see that all bacteria are excluded, and that the membranes are not permeable to protein, but will allow peptone to pass through. If the shells are used repeatedly, they must be scrupulously cleansed in order to avoid contamination from previous tests. By carrying out all proper precautions, the great majority of observers have obtained results agreeing with those of Abderhalden, the test proving positive in patients ranging from the early weeks of intra- or extra-uterine pregnancy to a few weeks post partum. Pearce and Williams (*Surgery, Gynecology, and Obstetrics*, April, 1918, p. 411) report a series of 86 cases of pregnancy giving positive results, controlled by negative results in a male and a non-pregnant female. Insufficient work has as yet been done using normal serum and serum of persons suffering with various diseases, to place the test on a conclusive basis. Pearce and Williams (*loc. cit.*) tested the reaction of serum of pregnant women with various organs, such as kidney, liver, and uterus, instead of placenta, and obtained positive results in some cases. While the test gives promise of becoming of importance in the diagnosis of pregnancy, yet further experiments to eliminate possible sources of error must be carried on before it will attain wide clinical application. In view of the present possible sources of error in the reaction, and par-

ticularly the difficulty of sending serum for a distance that is absolutely hæmoglobin-free, the advertisements of commercial laboratories that they are prepared to carry it out as a diagnostic measure are premature.

The optical or polariscopic method of reading the reaction, for which a polariscope is necessary, gives practically the same results as the dialysis-ninhydrin method, but at present entails so much expense that it is out of the reach of the ordinary laboratory.

Abderhalden and others are at present working on similar protein-splitting reactions for the diagnosis of cancer and other diseases, which promise much for the future.

Pearce and Williams have worked out a technic which promises to do away with many of the serious difficulties of the test, by which the reaction can be carried on in ordinary test-tubes instead of the diffusion shells. The mixture of serum and placenta is coagulated by heat and acetic acid, and the products of protein digestion, if present, are then separated from the coagulated serum proteins by filtration. Great caution must be observed that coagulation is complete and that the filtrate is rendered absolutely clear by a second boiling if necessary. This method does away in a large measure with the difficulties due to hæmoglobin-stained sera. We quote the following directions as to the technic from Pearce and Williams'

article: "Measured amounts of serum are placed in several tubes, and to one is added boiled placenta, to others boiled kidney, or heart, or whatever the control may be, and with one tube containing serum alone, and others the tissues named above, all are placed in the thermostat at 37° C. for 24 hours. At the end of this time the contents of each tube are poured into a separate beaker, diluted with 20 c.c. of water, boiled with the addition of acetic acid, and filtered. To 10 c.c. of each filtrate the ninhydrin test is applied."

Jamison and Cole (*New Orleans Med. Jour.*, lxvi, 8, p. 188) using Pearce and Williams' technic have confirmed its reliability as compared with the diffusion method.

SERO-ENZYME TEST FOR SYPHILIS

Baerslack (*Jour. Amer. Med. Assn.*, Mar. 28, 1914, p. 1002 and Aug. 15, 1914, p. 599) has applied the principles of Abderhalden's technic to the diagnosis of syphilis in a considerable number of cases. The tissues made use of as antigen in the reaction are the pearly white gummata resulting from the inoculation of the testicles of rabbits with syphilitic tissue. This tissue is prepared in the manner prescribed by Abderhalden for the placental tissue in the pregnancy test, and the remainder of the technic is the same as that of the Abderhalden dialysis method, using ninhydrin as indicator. Baerslack's results correspond

closely with those of the Wassermann reaction carried out simultaneously. In early cases of syphilis the sero-enzyme reaction was positive earlier than the Wassermann. In cases of tabes and paresis in which the cerebrospinal fluid was tested the reaction was never positive, showing that the enzyme is not present in the cerebrospinal fluid. The sero-enzyme test, moreover, was positive in cases of tabes in which the Wassermann reaction of the blood-serum was negative.

ABDERHALDEN-FAUSER REACTION IN MENTAL DISEASES

Fauser (*Münch. med. Wochenschr.*, Nov. 18, 1913, p. 884) applied the Abderhalden technic to the diagnosis of certain mental diseases, using as antigen tissue from the sex glands. In the case of males he employed testicular tissue, and in females ovarian tissue. According to his findings, the serum only of patients with dementia præcox contained protective ferments against these tissues, which caused him to regard the reaction as specific for the diagnosis of this disease. Later workers, however, have found that other mental diseases, such as paresis, manic depressive insanity and epilepsy, occasionally produced positive reactions. In a recent article, Simon (*Jour. A. M. A.*, May 30, 1914, p. 1701) concludes that a positive reaction, while not specific for dementia præcox as held by Fauser, is the rule in dementia præcox, while

it is the exception in purely functional psychoses, and that the test promises to become of great value when the technic, which at present is open to many errors, has been perfected.

MEIOSTAGMIN REACTION

Ascoli discovered that when a bacterial extract and the specific antibody produced by it in blood-serum were brought together a lowering of surface tension occurs, as shown by an increase in the number of drops in the fluid. The number of drops can be conveniently measured by Traube's stalagmometer. As an illustration, it may be found that a certain mixture of normal serum and extract of typhoid bacilli shows 50 drops; while a mixture of the same quantities of serum from a typhoid case and the extract of typhoid bacilli will show 52 drops. This phenomenon has been observed in the case of several diseases, including tuberculosis, anchylostoma, and echinococcus disease. For all these diseases, however, there are simpler clinical tests than the meiostagmin reaction. This reaction promises to be of some importance in the diagnosis of malignant tumors, particularly carcinoma. In this case the serum of the suspected patient is tested with an extract made from cancer tissue. It has also been found that an extract made from beef pancreas answers the purpose as well. An extract of the dried tumor tissue or pancreas is made

with methyl alcohol in the proportion of 1 : 4 at 50° C. for 24 hours. It is then filtered while hot, and again after cooling. The serum is employed in 1 : 20 dilution in normal saline solution.

In preliminary titration of the extract, decreasing quantities, made up to 1 c.c. with distilled water, are placed in tubes with 9 c.c. of 1 : 20 dilution of normal serum. A control is also prepared with plain water and serum (without extract). These mixtures are incubated at 37° C. for 2 hours, after which the number of drops in each tube is noted by means of the stalagmometer. For the test, the dose of extract is employed which is in the tube that contains 3-5 parts of a drop more than the control tube.

The following table will illustrate the titration of the extract:

				<i>Number of drops</i>	
9 c.c. normal serum (1 : 20) + 1 c.c. extract	1	50	Incubation 2 hours at 37° C.	59	+ 3 parts of a drop.
9 c.c. normal serum (1 : 20) + 1 c.c. extract	1	75		59	+ 1 part of a drop.
9 c.c. normal serum (1 : 20) + 1 c.c. extract	1	100		59	+ 1 part of a drop.
9 c.c. normal serum (1 : 20) + 1 c.c. extract	1	125		59	+ 1 part of a drop.
9 c.c. normal serum (1 : 20) + 1 c.c. extract	1	150		58	+ 9 parts of a drop.
9 c.c. normal serum (1 : 20) + 1 c.c. extract	1	200		58	+ 8 parts of a drop.
9 c.c. normal serum (1 : 20) + 1 c.c. extract	1	300		58	+ 8 parts of a drop.
9 c.c. normal serum (1 : 20) + 1 c.c. aqua dest. (control)				58	+ 8 parts of a drop.

In this case the dose of extract to be used in the test would be 1 c.c. of the 1 : 100 dilution, which shows about 3 parts of a drop more than the control.

Before using the extract for diagnostic purposes, this dose, as determined by titration, should be tested for reliability with a number of known carcinomatous

sera and known negative cases. If it proves reliable, it may then be used for diagnosis of unknown sera. Known positive and negative controls should always be used in performing the test, and titration of the extract should frequently be carried out, as its strength is not constant. A reaction is regarded as positive when the number of drops is more than $1\frac{1}{2}$ in excess of the control.

For the test, a mixture of the suspected serum and the dose of extract is made; also a mixture of the suspected serum and distilled water. The same thing is done with normal serum. These mixtures are then incubated for 2 hours at 37° C. In the case of a positive result the mixture of the patient's serum and tumor or pancreas extract must have an increase of more than $1\frac{1}{2}$ drops over the mixture of patient's serum and water, and over the mixture of normal serum and extract.

The following table gives an example of a positive result:

	<i>Number of drops</i>
Normal serum (control) 9 c.c. 1 : 20 dil. + 1 c.c. extract	
1:100 dil.	59
Normal serum (control) 9 c.c. 1:20 dil. + 1 c.c. aqua dest.	58 + 4 parts
Suspected cancer serum 9 c.c. 1 : 20 dil. + 1 c.c. extract	
1:100 dil.	61
Suspected cancer serum 9 c.c. 1:20 dil. + 1 c.c. aqua dest.	58 + 7 parts

Different investigators working with the meio-stagmin reaction give varying reports as to its relia-

bility. The general opinion appears to be that while it cannot be regarded as infallible in the diagnosis of carcinoma, yet it has a distinct clinical value when taken along with other tests and symptoms.

EPIPHANIN REACTION

The epiphanin reaction of Weichardt is based on practically the same principle as the meiostragmin reaction, viz., an acceleration of diffusion in the fluid when an antigen is brought in relation with the specific antibody. Seiffert has shown that this phenomenon is manifested by a change in reaction to phenolphthalein. This test has been applied especially to the diagnosis of syphilis. For this purpose 0.1 c.c. of a 1:10 dilution of the patient's serum is mixed with 0.1 c.c. of alcoholic extract of syphilitic fetal liver, 1 c.c. of decinormal sulphuric acid and 1 c.c. of an exactly equivalent solution of barium hydroxide are slowly added, making a neutral mixture. On the addition of a drop of an alcoholic solution of phenolphthalein, the fluid turns red if the serum is syphilitic, but does not change color if it is non-syphilitic. It is doubtful if this test will become as widely used as the Wassermann reaction for the diagnosis of syphilis.

XV

SPECIFIC BACTERIAL REACTIONS

ALLERGIC PHENOMENA—TUBERCULIN TESTS—LUETIN, GONORRHOEAL AND TYPHOID TESTS—SCHICK'S DIPHTHERIA TOXIN SKIN REACTION

THE interesting subject of hypersusceptibility, anaphylaxis or allergy has been sufficiently reviewed in Chapter IV. It will be recalled that allergy is merely an incident in the process of immunization and signifies simply the reactive changes exhibited by an individual after infection or the injection of some foreign substance or antigen. Allergic phenomena, therefore, may be characterized as those associated with increased sensitiveness (anaphylaxis), and those with diminished susceptibility (prophylaxis). It is apparent that a number of skin manifestations as erythematous and urticarial eruptions of serum sickness, exanthemata of eruptive fevers, "disposition to sudden cuticular inflammation" noted by Jenner in his studies of cow-pox in 1798, tuberculin reactions, etc., are pure allergic phenomena, since they are the exponents of localized foreign proteins and the toxic substances incident to their destruction by the specific antibodies in the blood of the sensitized body. Conspicuous among the allergic reactions to which practical consideration at this time will be given are the

tuberculin tests, the luetin test and the diagnosis of typhoid and gonorrhœal infection.

Tuberculins.—Tuberculin has been prepared in many ways and, although each preparation may be used diagnostically as well as therapeutically, it is true that one furnishes better results for diagnostic purposes, just as others are superior from the therapeutic standpoint. The available products include Koch's "old" and "new" tuberculins, Denys' "bouillon filtrate," Spengler's "perlsucht," Dixon's "bacillary extract," and Russian "tuberculinum purum."

Old Tuberculin ("O. T.").—The original tuberculin made and used by Koch has been styled "old" in contradistinction to his later or "new" tuberculins. It is prepared from the five or six weeks' pure culture of *B. tuberculosis* on five per cent. glycerin bouillon. The culture medium is then evaporated to one-tenth of its volume and filtered through porcelain. The filtrate containing all the soluble secretion products of tubercle bacilli is then diluted with glycerin, forming a twenty-five or fifty per cent. solution. The glycerin acts simply as a preservative, not as a germicide. Hence the stock solution may become contaminated if frequently opened, and it should be sterilized before making dilutions by heating on a water-bath for one hour at 60° C. or by boiling in a test-tube for ten minutes. It is customary when making

dilutions to use a solution of approximately 0.25 per cent. phenol in normal saline. It is advisable to make up fresh such dilutions about once in two weeks, as the phenol may cause some coagulation of the tuberculin, resulting in precipitation and deterioration. Dilutions showing marked sedimentation should be discarded.

New Tuberculin ("T. R.").—Realizing that old tuberculin was merely a toxin of tubercle bacilli, and that the immunity produced by it was to the toxin only and not to the bacterium itself, Koch, in 1897, seven years after the announcement of "O. T.," described a new tuberculin. This consisted of the residue (*Rückstand*) of tubercle bacilli and for brevity is known as "T. R." This tuberculin is prepared by drying *in vacuo* a virulent culture of tubercle bacilli. The dried substance is powdered in a mortar and triturated with normal saline. After centrifugation the supernatant cloudy fluid is discarded. The residue is again dried, ground, extracted with salt solution and centrifuged. This time the clean supernatant fluid is pipetted off and retained. The process is repeated until the residue is entirely used up. The clear solutions are united and added to glycerin, forming a twenty per cent. solution. "T. R." is standardized so that one cubic centimetre represents ten milli-

grammes of the original dried whole tubercle bacilli or two milligrammes of the active solid substance.

New Tuberculin ("B. E.")—Appreciating the fact that the injection of dead bacilli, as compared with "T. R.," caused an increase in the agglutination of the blood, Koch advocated a second new tuberculin, a bacillary emulsion (*Bacillen Emulsion* or *B. E.*). This is prepared by pulverizing finely a virulent culture of *B. tuberculosis*, and suspending one part of the powder in one hundred parts each of distilled water and glycerin. It is standardized so that one cubic centimetre contains five milligrammes of dried substance in suspension.

It will be borne in mind that the "new tuberculins" are suspensions, not solutions, hence they must be thoroughly shaken before use. Moreover, therapeutically, reactions are not so noticeable as after the use of "old tuberculin" and the resultant immunity is greater and more durable, both of which have added to their popularity.

Denys' Tuberculin ("B. F.")—In 1905 Denys recommended the filtrate from bouillon cultures of tubercle bacilli. This tuberculin is commonly known as Bouillon Filtrate or "B. F." It is essentially the same as Koch's old tuberculin, differing only in that no heat is used in its preparation. It contains all the normal soluble products of the *B. tuberculosis*.

Spengler's Perlsucht Tuberculin ("P. T. O.").—*Perlsucht* signifies "pearl disease" in cattle, that is, true bovine tuberculosis. Spengler believed that a tuberculin prepared from this strain of the tubercle bacillus would prove most efficacious in the treatment of human tuberculosis, and indeed such was the case in his experience. The method of preparation of Spengler's tuberculin is precisely identical with that of Koch's old tuberculin.

Dixon's Tuberculin.—This product is a saline extract of living tubercle bacilli minus their fat. Six to eight weeks old cultures from four per cent. glycerin veal broth are removed and collected on hard filter paper. Equal quantities, by weight, of human and bovine types of bacilli are placed between sterile filter paper and dried in a thermostat for twenty-four to forty-eight hours. The dried bacteria are then treated in an excess of ether until all water and glycerin are removed. Further extraction of the fat with fresh ether is done and the same is removed from the bottom of the vessel by a Pasteur pipette. After the bacillary mass has been thoroughly dried and freed from ether, it is ground in an agate mortar and suspended in normal saline in the proportion of parts one to five. The suspension is then shaken for eight to ten hours, after which it is allowed to stand at room temperature for several days. Finally it is filtered until the filtrate is

free of bacilli, as determined microscopically, culturally and by animal inoculation. One cubic centimetre of this extract represents one-half gramme of the bacillary mass. One-half per cent. phenol is added as a preservative.

Tuberculinum Purum ("T. P.").—According to "New and Non-official Remedies," this Russian tuberculin is "the purified filtered extract of human tubercle bacilli in 50 per cent. glycerin, prepared in the same way as in Koch's old tuberculin, but subsequently treated with alcohol, ether, chloroform and xylol in order to remove deuterio-albumoses." These toxalbumins and glycerin-soluble by-products of the culture medium are held responsible for a certain amount of the toxic reaction noted in using old tuberculin and their elimination has resulted in this so-called purified tuberculin.

TECHNIC OF MAKING DILUTIONS

Many pharmaceutical firms to-day market tuberculins in serial dilutions most convenient for immediate use. However, if it be desirable or necessary to employ stock preparations, the following technic may be found useful:

Pipettes.—(a) One-tenth c.c. pipette, graduated in hundredths, is most economical of stock in making tuberculin dilutions.

(b) One c.c. pipette, graduated in tenths for larger dilutions.

(c) Ten c.c. pipette, graduated in tenths of a c.c. for the highest dilutions.

Pipettes are best sterilized by dry heat in specially constructed copper containers, or they may be kept immersed in a jar of two per cent. phenol or alcohol, rinsing in sterile diluting solution before use.

Dilutions.—A convenient method of making dilutions economically is as follows:

Dilution No. 1: 0.1 c.c. stock tuberculin, pipette (a) + 9.9 c.c. diluting solution = 0.001 c.c. tuberculin.

Dilution No. 2: 0.1 c.c. of Dilution No. 1, pipette (b) (1 subdivision) + 9.9 c.c. = 0.1 c.c. = 0.00001 c.c. tuberculin.

Dilution No. 3: 0.1 c.c. of Dilution No. 2, pipette (b) (1 subdivision) + 9.9 c.c. = 0.1 c.c. = 0.0000001 c.c. tuberculin.

In doses of 0.001 c.c. and over it is advisable to use pipette (b) for measuring the stock tuberculin.

The Physiological Action of Tuberculin.—Many theories have been advanced. The most tenable appears to be that of Citron, who explains the presence of antituberculin, demonstrated by Wassermann, Bruck and Ludke, in the bodies of tuberculous subjects, on the assumption that after an injection of tuberculin the cells in the immediate vicinity of the

tuberculous focus unite with tuberculin by their receptors (Ehrlich), and the cells thus attacked produce receptors in excess of the demand. These over-produced receptors or antibodies are then set free in the serum to unite with other portions of tuberculin. Thus repeated tuberculin inoculations lead to the formation of large numbers of free agglutinins, anti-tuberculin and opsonins at the point of local infection as well as many fixed receptors or antibodies.

Obviously, it will be seen that the tuberculin reaction is dependent upon the presence of specific antibodies. If the suspected individual is free from tuberculosis and none exists, no reaction can be produced even by recourse to very large doses of tuberculin; nor can a reaction be elicited in the advance stages of the disease, because all antibodies have been consumed. On the other hand, in the average case of tuberculous infection, a comparatively small dose of tuberculin will suffice to evoke a reaction. The typical tuberculin reaction is threefold: general, focal and local. The *general reaction* consists of malaise, headache, insomnia, bodily aches, nausea, cough, tachycardia, and particularly a rise in temperature of one or more degrees. These phenomena are probably due to the fact that tuberculin, like any other protein, is split up by complement acting in conjunction with antibody, and the split toxic products formed give rise to the symp-

toms as they are eliminated. The *focal reaction* consists of the fresh inflammatory changes noted at the tuberculous focus, namely, congestion, pain, tenderness, swelling, redness, etc. The *local reaction* comprises the inflammatory signs observed at the site of the injection. The focal and local reactions are explained by the interaction of combined tuberculin, newly formed antibodies and complement, in attracting phagocytes, with direct localizing action, producing thereby an inflammatory reaction. If the local reaction be severe, necrotic tissue may be cast off. Following the above reactions, there is a tendency exhibited by the pathological process to heal.

Tuberculin as a Diagnostic Agent.—The employment of tuberculin in a diagnostic capacity is very extensive, and rightfully so, because, properly and competently utilized, its value at times as an aid in differential diagnosis is inestimable. In the authors' experience the positive or negative information thus yielded has been absolutely dependable. Its proper use, however, entails the greatest caution and discrimination as to indications and contra-indications on the part of the patient, size and administration of doses, and, not least of all, the correct brand of tuberculin. Unanimity of opinion prevails that Koch's old tuberculin, "O. T.," whatever may be its method or form of application, is best for diagnostic purposes.

The various methods utilized for the application of tuberculin diagnostically comprise, (1) subcutaneous injection, (2) intradermic injection, (3) cutaneous scarification, (4) percutaneous anointment, and (5) mucous membrane instillation.

Method of Subcutaneous Injection.—First practiced by Koch, it supersedes in reliability any other method of tuberculin application. Carelessness may render it the most dangerous method, but properly carried out it is absolutely harmless. It has been variously modified as to size and interval of dosage. Old tuberculin (O. T.) is the preparation of choice. Koch stipulated that doses up to 250 milligrammes could be administered to perfectly normal individuals without reaction, but advised a limit of 10 to 25 milligrammes in practice. We feel that such doses are entirely too large, and, with few exceptions, agree with Roth-Schultz respecting the technic of inoculations. The subcutaneous tuberculin test is best carried out with the patient in bed or at rest. His temperature and pulse should be recorded every two or three hours for two or three days, also all clinical signs and symptoms must be noted prior to starting the first inoculation. The primary injection given is 0.5 milligramme. Should the slightest indication of a reaction, either general, focal, or local (see page 174), supervene, the same sized dose is to be repeated two or three days

after its subsidence. If no sign of a reaction occurs, the dose may be increased to 1.25 milligrammes on the third day. Again if the typical reaction is not produced and merely suggestive signs appear, the same sized inoculation is repeated, since this may be sufficient to provoke a marked reaction after the previous sensitization. In the absence of any reactive phe-

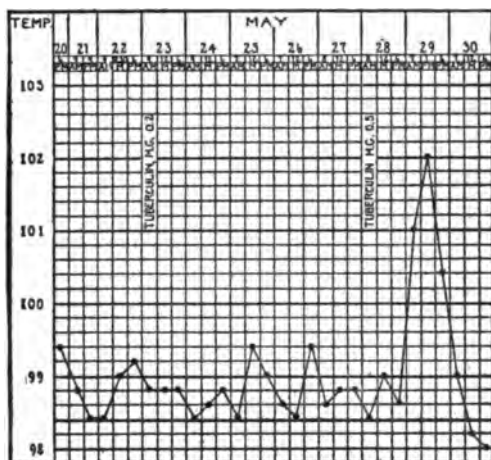


FIG. 15.—Edward G., aged 77. Tuberculous mastoiditis. Note typical diagnostic tuberculin reaction after second injection of tuberculin. Associated with the febrile rise and general reactions of malaise and headache, was a local reaction of increased pain and discharge from the mastoid.

nomena after three or four days, an injection of 2.5 milligrammes should be given. If this fails to cause a definite response, a final maximum inoculation of 5 milligrammes is permissible. In children, under fifteen years of age, Baldwin recommends doses of 0.05, 0.2, 0.5 and 1 milligramme. A reaction to be pathognomonic for any suspected lesion must comprise, in

addition to a rise in temperature of at least one degree, increased focal inflammatory signs, located in the lungs, bones, joints, epididymis or wherenot (Figs. 15 and 16). Considerable importance is to be attached, also, to the inflammatory areola oftentimes surrounding the site of injection (local reaction). Ob-

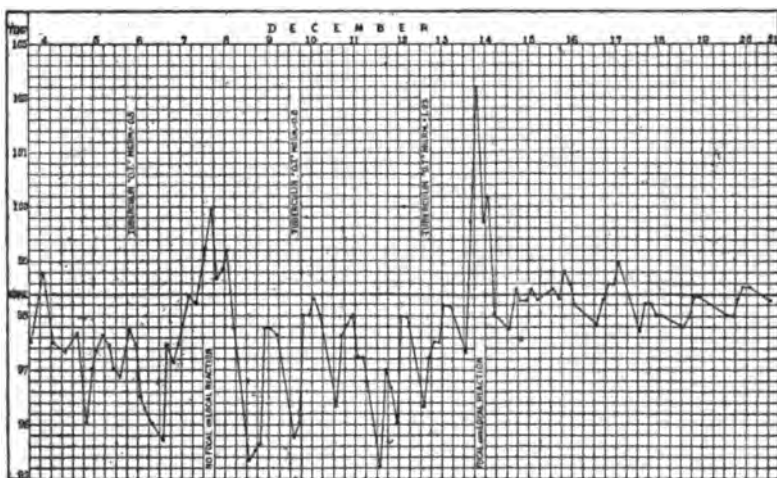


FIG. 16.—R. W. S. Bilateral tuberculous epididymitis. Observe the suggestive rise in temperature after the first injection of tuberculin, not to be regarded, however, as pathognomonic, owing to the absence of any focal reaction. The reaction occurring on the thirteenth of the month is typical generally, focally and locally.

viously, a focal reaction outweighs in significance both the general and local reactions, and under such circumstances a positive test may be pronounced even though the febrile rise has not amounted to a degree. The sites commonly chosen for the injections are the outer or posterior aspects of the arm, the supra- or interscapular areas, the outer aspects of the thigh or

leg and the lumbogluteal regions. After cleansing the skin with alcohol, the needle is to be introduced well subcutaneously and care taken not to pierce the muscular sheaths or to enter a vein. The syringe best adapted for making the inoculations is an all-glass type graduated in minims or, better, fractions of a cubic centimetre, owing to its cleanliness and ease of sterilization (Fig. 17). Reliable pharmaceutical houses supply tuberculin "O. T." in serial dilutions ready for immediate use. If the above technic be carefully followed, and the patient restricted to bed on the



FIG. 17.—All-glass tuberculin syringe, graduated into hundredths of a cubic centimetre, particularly useful for both diagnostic and therapeutic inoculations.

occurrence of a reaction, until its disappearance, the test is devoid of danger, even when incipient and latent pulmonary tuberculosis is present.

The subcutaneous tuberculin test, as stated above, is indicated in all forms of localized tuberculosis, when the diagnosis is in doubt. Among these lesions may be enumerated tuberculous ulcerations of the skin, lymphadenitis, osteitis, synovitis and arthritis, laryngitis and tracheitis, phlyctænular conjunctivitis, keratitis, iritis, uveitis, nephritis, prostatitis, seminal vesiculitis, epididymitis, peritonitis and pulmonary tuberculosis.

The contra-indications to the use of the subcutaneous tuberculin test may be stated to be general miliary tuberculosis, acute phthisis, advanced pulmonary tuberculosis, tuberculous meningitis, markedly asthenic individuals and convalescents from an acute illness, conditions in which any tuberculin application may fail to produce reaction; in patients running a marked irregular temperature, and possibly also in children, the scarification or von Pirquet test takes precedence over the method of subcutaneous injection.

Intradermic Injection.—Mantoux has strongly recommended this method for the administration of tuberculin, diagnostically, and to determine, by the severity of the reaction, the sensitiveness of the patient prior to therapeutic inoculations. He claims that it is more sensitive than other tuberculin tests. Indeed it would appear too delicate, as almost all adults show a reaction, although tuberculosis, clinically, is absent. The test may be serviceable in very young children, but is not destined to become popular. Mantoux employs 0.01 milligramme injected into the skin of the thigh. The reaction is not unlike the local inflammatory process observed in the subcutaneous method.

Scarification or von Pirquet Cutaneous Reaction.—This test, like the subcutaneous method, depends upon the antituberculin in the body fluids of the in-

fectured individual. The reaction is very delicate, thus accounting for the high percentage of positive results. The test has its greatest applicability in children and patients exhibiting a comparatively high fluctuating temperature. Old tuberculin ("O. T."), again, is the preferable preparation in the performance of von Pirquet's test. It is conveniently put up in sealed capillary tubes and marketed by a number of pharmaceutical firms. The technic is quite similar to vaccination against smallpox. An area on either the arm or leg, usually the forearm, is cleansed with ether, *not alcohol or soap and water*, and a drop of "O. T." is placed on the skin; about two inches distant a second drop of glycerin bouillon, utilized as a control, is placed. With a lance, a needle or special scarifier, immersed first in the central drop, then the drop of tuberculin, the epidermis is lightly scratched, removing only the superficial epithelium and avoiding, if possible, any evidence of blood. The drops should be allowed to evaporate to dryness, before applying a dressing of sterile gauze, otherwise they may be covered with a vaccine shield. At most a very slight hyperæmia, disappearing in twenty-four to forty-eight hours, due to traumatism, may be noted at the control point. A typical positive reaction at the site of the tuberculin drop is characterized by a hyperæmic and inflammatory area from four millimetres to three

centimetres in diameter. The intensity of the reaction varies from a hyperæmia with papule formation to many papules on an œdematous and inflamed base, to a markedly indurated zone exuding serum from the scarification site (Plate III). The inflamed area may persist for a week or two. The reaction is not attended with general fever or other symptoms. A positive test simply indicates the presence of tuberculosis; it does not specify, as does the focal reaction in the subcutaneous test, that a suspected joint, cornea, epididymis, prostate, etc., is tuberculous. A patient with tuberculous peribronchial lymphadenitis may exhibit a positive reaction, while his particular cause for complaint, a troublesome knee, may be gonorrhœal.

Detre had evolved a modification of the above, known as a *differential tuberculin test and therapeutic control*. It has for its objects the differentiation of the human and bovine types of infection, the measurement of the extent of the disease, whether incipient or advanced, the selection of the best variety of tuberculin for immunization and a control in therapy superior to the opsonic index. Three varieties of tuberculin are employed: Koch's "O. T.," "B. F." prepared from bacilli of the human type, and "B. F." as in the von Pirquet method, preferably the flexor surface of the forearm is cleansed with ether and a pledget of

PLATE III



Von Pirquet's cutaneous tuberculin test (positive reaction).

cotton. At distances of about three inches, from above downward, drops respectively of commercial "O. T.," human "B. F." and bovine "B. F." are expressed on the skin from capillary tubes. In the medium of each drop the underlying skin is scarified, care being taken to cleanse the scarifier in passing from one variety of tuberculin to the other. The inoculated sites should be inspected every twelve to twenty-four hours. If no reaction takes place in three days the test may be pronounced negative. Positive reactions differ in no respect from those described under the von Pirquet test. Detre points out that a greater reaction at the site of inoculation with the bovine tuberculin means tuberculosis of that type and indicates the use of tuberculin of the bovine type in therapy; also that if greater reactions occur with the Denys filtrates than with "O. T.," Koch's old tuberculin should be employed in immunization and *vice versa*. Marked reactions are usually observed in early virulent infections, although not infrequently they occur in the chronic latent form of the disease, including surgical cases. The reaction to filtrate is prone to disappear more in old chronic cases. Routine periodic applications of the test are necessary if it is to be utilized as a control of therapy.

Method of Percutaneous Anointment.—Moro describes the use of an ointment compounded of equal parts of tuberculin "O. T." and refined anhydrous

lanolin. The test is not so delicate or reliable as either the premier subcutaneous method or even von Pirquet's scarification, but is utilized by some practitioners, especially in febrile patients, because of its ease of application and absolute harmlessness. The ointment is applied as follows: An area of skin about five centimetres in diameter on the abdomen or in the mammary region is cleansed with soap and water, alcohol and finally with sterile water to remove all traces of alcohol. A mass of the ointment, about the size of a pea, is thoroughly rubbed into the prepared area for one-half to two minutes. A gauze dressing, covered with oiled silk or wax paper, is serviceable to protect the patient's clothing. The reaction, occurring usually within a few to forty-eight hours, rarely delayed for a week, is characterized by a few to a hundred or more discrete papules from one to five millimetres in diameter, in the case of marked reactions surmounting an erythematous base and associated with itching, at other times not. The papules dry up and desquamate in a few days and at the end of two weeks merely a brownish pigmentation remains visible. The reaction is not accompanied with fever, pain or undue discomfort. The more intense reactions are observed in scrofulous conditions and bone tuberculosis; weaker reactions are usually seen in infections of the lungs.

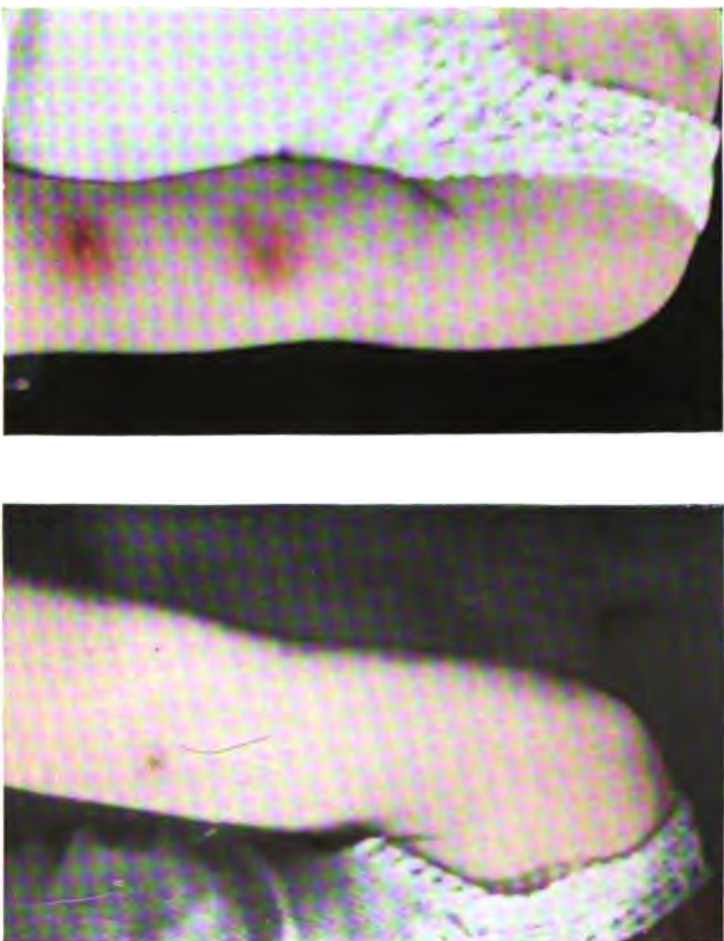
Method of Mucous Membrane Instillation.—Wolff-Eisner first reported on the use of ten per cent. "Alttuberculin" as a valuable diagnostic test when instilled into the conjunctiva. Almost synchronously, Calmette pointed out the irritation due solely to the contained glycerin in ordinary tuberculins, especially "O. T.," and recommended its application as an ophthalmic test only after precipitation and purification with alcohol. Even as such the conjunctival test has not been received in all quarters with much favor, owing to a number of serious ophthalmic complications, which have arisen incident to the use of tuberculin in the eye. Therefore, it is strongly contra-indicated in conjunctivitis, ulcers, blepharitis, iritis, keratitis, trachoma and all diseases of the internal structures of the eye. Scrofulous persons are predisposed to the formation of phlyctenules as a result of the reaction. Tuberculin for this test is obtainable, commercially, in either solution or tablet form. Baldwin advises an initial instillation into the conjunctival cul-de-sac of one minim of a one-half per cent. solution; in the absence of a reaction in forty-eight hours, the same quantity of a one per cent. solution is instilled into the *other* eye. The reaction usually develops in three to twelve hours, but may be delayed a day or two, and often endures for a week. It is characterized by itching, burning, serofibrinous exudate, congestion and

redness of the caruncle, sometimes involving the entire palpebral conjunctiva and lids.

Vaughan, Jr., states that he has never seen any ill effects as a result of the application of the test. His technic consists in dissolving a tuberculin tablet in five minims of boiling hot water, producing a one per cent. solution. One drop of this, after cooling, is instilled into the conjunctival sac; if no reaction takes place, at the end of a week a second instillation of one drop of one-half per cent. solution is instilled into the same eye. He believes the zymogen created by the first instillation is activated by the second, causing the anti-tuberculin ferment to attack the tuberculin, thereby liberating the toxic cleavage product in large amount, even though the second dose is but one-half of the first. As soon as a reaction becomes distinctive, the conjunctiva is copiously flushed with a solution of boric acid, in order to prevent undue inflammation. This tuberculin test has been unsuccessfully applied to the nasal and vaginal mucosa.

Luetin Cutaneous Reaction in Syphilis.—Noguchi, in 1911, described an allergic cutaneous reaction in syphilis. Following the successful cultivation of the *Treponema pallidum* (*Spirochæta pallida*) in ascitic fluid and ascitic fluid agar containing pieces of placenta, the agar growth was ground to a paste in a mortar, then diluted with the fluid culture, sterilized

PLATE IV.



Luetic cutaneous reaction. Left arm demonstrates the papular character of the reaction on an erythematous, indurated base. Right arm shows the negative control. (Noguchi.)

at 60° C. for an hour in a water-bath, preserved in 0.5 per cent. phenol and standardized with the dark-field microscope, so that forty to one hundred dead treponemata occupied the average field. To this preparation Noguchi assigned the name *luetin*, and has recommended it as a valuable diagnostic aid, superseding even the Wassermann reaction in certain forms of syphilis. The technic of the test consists in sterilizing a small area of skin of both arms, using alcoholic sublimate solution. Intradermically, in one arm, with a syringe having a very fine needle, 0.05 of a cubic centimetre of a control suspension is injected; in the opposite arm a similar quantity of luetin.

Normal or negative reactions consist of a small erythematous and occasionally a papular formation, not attended with pain or itching sensation, occurring in 24 hours, and disappearing usually in 48 hours and always in 72 hours, even if papular. No induration results, although yellowish pigmentation due to ecchymosis is commonly seen.

Positive reactions assume three forms: (1) *papular*, (2) *pustular* and (3) *torpid*, and are characterized by the formation, a day or two after inoculation, of a large, reddish papule usually five to ten millimetres in diameter (Plate IV). A hyperæmic zone frequently surrounds the papule. The inflammatory process increases and extends for the next three or

four days, then slowly recedes, all signs disappearing usually in a week or two. At the height of the reaction the papule commonly presents a bluish-red coloration and may be associated with vesicles, even undergoing pustulation and ulceration with secondary crust formation. In the so-called torpid form, the slight reactive signs entirely disappear in three or four days and will suggest a negative result. However, after ten or more days, the injected site may light up and proceed to pustulation as above described. Systemic reactions, as malaise, loss of appetite, diarrhoea and slight rise of temperature occur rarely.

It is not assumed that this test will replace the Wassermann reaction. Certainly, the Wassermann reaction is infinitely superior to the luetin test in the primary, secondary and acute tertiary stages of syphilis, although in the latent nervous and hereditary forms of the disease Noguchi adheres to the superiority of the skin reaction. He found the test positive in 100 per cent. of manifest tertiary lesions, in 94 per cent. of latent tertiary and in 96 per cent. of hereditary syphilis. It would seem that the serological reaction is more readily influenced to become negative under treatment than is allergic cutaneous reaction.

Cutaneous Reaction in Gonorrhœa.—In 1908, Irons alluded to the diagnostic value attached to the constitutional disturbances provoked by therapeutic

PLATE V.



Gonorrhoeal allergic reaction, demonstrating the papulo-erythematous cutaneous response on the third day after the intradermic injection of one cubic millimetre of a killed polyvalent suspension of gonococci.

inoculations of dead gonococci and suggested the use of gonococcic bacterin in this capacity. At the time of this report, one of the authors (Thomas) had been utilizing an alternation of subcutaneous injections of tuberculin, gonococcic, streptococcic, pneumococcic and staphylococcic bacterins in the differential diagnosis of the etiological bacterium in obscure articular affections, with a view to the determination of the correct biological therapeutic agent. Contrary to Irons' apparent greater reliance on the general and focal reaction, Thomas attached more significance to the local reaction at the injectional site, employing much smaller doses, 100,000,000 instead of 500,000,000 cocci. Recently the substitution of unheated bouillon filtrate or a filtrate similar to old tuberculin for the bacterin has produced more dependable results. Whether the filtrate or bacterin is used in small or large dosage, subcutaneously or intradermically as has been done by London, or by scarification, the reaction to be looked for simulates precisely that observed in tuberculin tests, namely, local hyperæmic areas with or without papules or crusts, focal inflammatory changes at the affected site, malaise, leucocytosis, rise in temperature, etc. The reaction appears in six to twenty-four hours and persists for two to three days, usually unattended by systemic phenomena, unless large doses are administered. No control is necessary, as relatively

large inoculations of dead gonococci do not produce the reaction in non-gonorrhœal subjects. The test finds its greatest field of usefulness in the differential diagnosis of obscure synovial, joint, periosteal and gynæcological affections; also promises to be of value in the control of candidates for matrimony and as a guide to the insufficiency of treatment.

Cutaneous Reaction of Typhoid Immunity.—Gay and Force very recently have announced a decisive skin reaction in 95 per cent. of persons who have had typhoid fever. Furthermore, they found that nine out of fifteen individuals inoculated from four and three-fourths years to eight months previously, in accordance with the method of the United States Army, gave positive reactions, and that of twenty-five persons inoculated within eight months, none showed a negative response. They conclude that the test may serve as an index of the duration of typhoid immunity following protective inoculation, thereby governing the time for re-inoculation.

The preparation used for the test consists of typhoid cultures grown for five days in five per cent. glycerin-broth. This is evaporated to one-tenth of its original volume over acetone, simulating the preparation of Koch's old tuberculin. The test is carried out precisely as is von Pirquet's tuberculin method, producing reactions of identical character (see page 180).

SCHICK'S DIPHTHERIA TOXIN SKIN REACTION

This test, which promises to be of great practical importance, has recently been studied in this country by Veeder (*Am. Jour. Dis. Child.*, 1914, viii, 154-162). The technic is similar to that of the Noguchi-luetin reaction, and consists of the intracutaneous injection of an amount of diphtheria toxin equal to 1-50 of the lethal dose for a 250-gramme guinea-pig. This is so diluted that the amount of toxin is contained in 0.1 c.c. of fluid. A positive reaction is characterized by an area of redness and swelling within 24 or 48 hours, and indicates the absence of antitoxin in the blood. A negative result, on the other hand, is evidence that the individual is well supplied with diphtheria antitoxin. By actual test it has been found that in the case of a negative result the individual tested possesses at least 0.081 unit of antitoxin per cubic centimetre of blood, which is considered sufficient to protect him from an ordinary attack of diphtheria.

The practical importance of the test lies in its use as a preliminary indicator of the necessity of protective injections of antitoxin in persons exposed to diphtheria. It is argued that those giving negative reactions already possess in the blood sufficient antitoxin for protective purposes, and therefore require no prophylactic injection. Those giving positive reactions, on the other hand, possess insufficient antitoxin,

and require protective inoculations. Veeder's studies show that in children from birth to 15 years of age, which includes the period of greatest liability to diphtheritic infection, approximately 50 per cent. give negative reactions. If the test proves its reliability, this will mean a saving of one-half of the amount of antitoxin to be used prophylactically, and also a great lessening in the number of cases of temporary disability that sometimes follows antitoxin injections.

Kolmer and Moshage (*Am. Jour. Dis. Child.*, March, 1915, p. 189), after extended studies with this reaction, reach the following conclusions:

1. The toxin skin reaction is a valuable and reliable method for detecting susceptibility to diphtheria.
2. Persons reacting negatively to this test usually contain at least $\frac{1}{10}$ unit of diphtheria antitoxin per cubic centimetre of serum, and this amount of antitoxin is probably sufficient to protect against infection.
3. Persons reacting weakly or strongly positive usually contain less than $\frac{1}{10}$ of a unit of antitoxin per cubic centimetre of serum or none at all. They may be regarded as susceptible to diphtheria and in the event of exposure to infection should be passively immunized with antitoxin injection.
4. About 40 to 50 per cent. of children ranging from 1 to 15 years of age react positively to the toxin test; this means that the preliminary use of the toxin test will eliminate the necessity of administering prophylactic doses of antitoxin to about 50 per cent. of children.
5. The toxin reaction indicates that the immunity conferred by an injection of antitoxin begins to disappear after ten days and has generally passed away entirely after four weeks.
6. The increased susceptibility of persons with scarlet fever to diphtheria is shown by the toxin reaction; even after the injection of antitoxin about 10 per cent. are susceptible within ten days.
7. According to the toxin reaction the immunity conferred by an attack of diphtheria is usually of short duration or entirely absent.
8. The most practical application of the toxin reaction consists in applying the test as a preliminary measure to all persons who have been exposed to diphtheria and immunizing only those who react positively.

XVI

TUBERCULIN THERAPY

PROPHYLAXIS—THERAPEUTIC ADMINISTRATION OF TUBERCULIN—AVAILABLE PREPARATIONS—MODES OF ADMINISTRATION AND DOSAGE—CONTROL OF TUBERCULIN TREATMENT—LIMITATIONS AND CONTRA-INDICATIONS—INDICATIONS AND RESULTS

Prophylaxis.—Although the promises of Koch, Behring, Maragliano, Arloing, Friedmann and others have never measured up to their greatest expectation, hope still exists in the breasts of investigators that the future holds a specific for inoculation against tuberculosis. Indeed, there appear to be good reasons, both experimentally and clinically, why this should become a realization. Auto-immunization in human beings is probably of common occurrence. This is attested to by the prevalence of tuberculosis in childhood, and the fact that only 25 per cent. of patients succumb to the disease, while in the remaining 75 per cent. almost all exhibit evidence of healed tuberculous foci. Thus it is logical, irrespective of the assertion that tolerance to tuberculin probably never means the production of true immunity in tuberculosis, that we should reinforce our defensive forces, prophylactically as well as therapeutically. Indeed, Hamburger, in view of the frequent contraction of tuberculosis in early childhood, advises immunization in infancy and asserts that the proper administration of tuberculin at

this time constitutes one of its most valuable uses and will confer immunity.

Therapeutic Administration of Tuberculin.—Although tuberculin has failed to become the much-vaunted panacea that, at first, it was hoped would be realized, it, at least in most cases, when properly employed, favorably and decidedly influences the tuberculous process. This has led, in recent years, to a marked revival of interest in its therapeutic employment.

It must be clearly and definitely understood by the clinician, as a fundamental premise, that it is not claimed that tuberculin is a specific for, or *per se* a curative agent in, the treatment of tuberculosis. It is to be regarded simply as an accessory agent of Nature, and when thus utilized serves as a most valuable, if not indispensable, adjunct to routine antituberculosis measures, amounting frequently, in localized and non-febrile cases, apparently to curative results. Failure is due oftener to incompetent, careless, or reckless administration than to tuberculin itself. It must be constantly borne in mind that tuberculin, more so than most biological products, is a powerful agent, capable of producing evil, or even disaster, rather than good, unless properly and wisely administered. In experienced hands, however, tuberculin therapy is absolutely harmless. Trudeau states:

We have learned the dangers of tuberculin treatment and its evident limitations. We have, however, also in late years learned something about the complex defensive resources of the living organism which tend to the production of immunity, and how to call them into action, though we are evidently as yet only on the threshold of the knowledge of immunization by vaccines in the treatment of chronic infections. Everything we know, however, points to immunization as the goal toward which our efforts should be directed. We have much to learn about tuberculin treatment, but even in the present state of our knowledge, I am inclined to think that the production of tuberculin immunity by the mild clinical method is capable of favorably influencing the course of chronic tuberculosis, of prolonging life, and in many cases of aborting a commencing infection or extinguishing the smouldering fires of a chronic infection.

Available Preparations.—It is obvious, from the great number of tuberculins that have been recommended during the past twenty-five years, that none is ideal, particularly for therapeutic purposes. Some have fallen into disuse because of their greater toxic properties; others have gained confidence because of greater immunizing power; still others have been subjected to purification processes, in the hope of eliminating toxic reactions and so on. Those preparations which to-day enjoy greatest popularity, in the order named, are Bacillen Emulsion or "B. E.," Tuberculin Rückstand or "T. R.," Bouillon Filtrate or "B. F.," Old Tuberculin or "O. T.," Spengler's Perlsucht Tuberculin, "P. T. O.," Dixon's Bacillary Extract, and Tuberculinum Purum, "T. P." (see Chapter XV). The majority of tuberculin therapists favor Koch's so-called new tuberculins, "B. E." and "T. R.," believing that the resultant immunity is

greater and the toxic effect is less; some still adhere to "O. T." or its bovine preparation according to Spengler; a few have faith in Dixon's product and many have been impressed by the virtue of "T. P.," which Neumann has shown to be the least toxic, hence to be preferred in febrile cases or when the process of immunization is to be effected in the shortest possible time. The ideal *modus operandi* would appear to be a combination or alternation of "B. E." and "B. F.," the former consisting of the bacilli and their extractives inactivated by a minimal degree of heat, the latter comprising the unheated filtered toxins of the bacilli; thus the patient would seem to be immunized against all constituents of the bacillus. A number of attempts have been made to employ so-called modified tubercle bacilli in the treatment of tuberculosis. The most recent failure of this nature was the well-advertised "Friedmann Cure" or inoculation with non-virulent turtle tubercle bacilli.

Modes of Administration and Dosage.—The usual method of administering tuberculin to patients is by *subcutaneous inoculation* (for technic see Chapter XV, p. 178). In the case of "B. E." and "T. R.," the common initial dose may be 0.001 milligramme;¹ with Dixon's tuberculin, the beginning inoculation

¹ A few clinicians utilize and recommend the superiority of infinitesimal doses in tuberculin therapy, namely, an initial inoculation of 0.0000001 to 0.00000001 milligramme of "B. E." or "O. T."

consists of the extract from 1.0 milligramme of tubercle bacilli; with *Tuberculinum Purum* or "T. P.," the usual primary dose is 0.02 milligramme. In the *absence of reactions*, the size of these doses is doubled, semi-weekly and later weekly, until the maximum tolerant dose is reached. Dixon advises that the minimal dose be repeated five times at intervals of five days, before proceeding to the next higher dose, which is ten times the strength of the first. This is repeated five times before giving dilution No. 3, which is twice the strength of the former. Thus the patient is carried through seventeen more dilutions, each being an increase of one-tenth of its predecessor. In children the size of the dose is regulated by the age of the patient, as in any other therapeutic remedy. As a rule children tolerate tuberculin very well.

For most tuberculins the technic of making dilutions as described in the previous chapter on page 172 will prove satisfactory. Pharmaceutical firms now prepare various tuberculins in serial dilutions, bearing legends explanatory of their use, and rendering their employment a matter of great convenience. If the dilutions are not freshly prepared, caution must be exercised to see that they are not clouded or precipitated, hence inert, due to the action of the preservative phenol. Tuberculins for subcutaneous inoculation are thus marketed in vials with hermetically sealed rubber

caps, glass ampoules, or in tablet form. Tuberculin tablets, "B. E." and "T. R.," are now obtainable in six strengths, namely, 0.0001, 0.001, 0.01, 0.1, 1.0 and 10 milligrammes. By dissolving a tablet in one c.c., using a special graduated syringe (Fig. 17), or in a ten c.c. graduate containing ten c.c. of water or fractions thereof, injecting never more than one c.c., the inoculations with proper sized dosage can be readily executed. Tablets possess the advantage of being more stable than solutions, although great precautions as to sterility must be observed.

Contrary to the researches of Pfeiffer and Persch, that pepsin, trypsin and enterokinase destroy the activity of tuberculin, a number of investigators have administered and recommended *tuberculin by mouth*, stating that when thus administered it is just as effective as by subcutaneous injection, and carelessly given is equally capable of harm and even fatal results. Oral treatment has been carried out with 0.00001 to 0.001 milligramme of "T. R." in 10 c.c. of normal horse serum; in the presence of mixed infection, staphylococcic bacterin has been combined with the dose, best given on an empty stomach. Pharmacologists have prepared triturates of "B. E." and "T. R." for mouth administration, the tablets arranged serially and containing from 0.000001 to 0.01 milligramme each.

Tuberculin has also been given *per rectum* either in dosage of 0.001 milligramme "T. R." in normal serum or in the form of suppositories as recommended by Lissauer. Suppositories of "O. T." are obtainable, the doses ranging from one to five hundred milligrammes.

Tuberculin therapy by mouth, and to a less extent by rectum, thus far has not been and probably never will be very favorably received by the profession.

The *physiological action of tuberculin*, locally, focally, and generally, has been sufficiently described in the foregoing chapter (see page 173).

Control of Tuberculin Treatment.—Specific therapy under no circumstances invites or permits relaxation in the general hygienic and dietetic management of the case.

Tuberculin therapy may be controlled by (1) the opsonic index or (2) the clinical symptomatology.

The *opsonic control of Wright* (see Chapter XX) has comparatively few adherents in the treatment of tuberculosis, although Wright and his school have found it particularly well adapted to govern the inoculations in surgical tuberculosis. They believe that so long as the opsonic index remains in the positive phase, further and particularly increased dosage is unnecessary and inadvisable.

The *clinical symptomatology* serves as the popular

method in the control of tuberculin therapy, not only because of the tedious technic of the opsonic index, but also on account of the inferior results of the latter method, at least in the pulmonary form of the disease.

The practitioner should not be led to believe that tuberculin treatment, properly guided by the clinical symptoms, is an easy matter requiring no special instruction or knowledge. Far better that he realize that tuberculin is a double-edged sword, cutting success on the one hand and strewing disaster on the other. Due consideration and correct interpretation of trivial symptoms, as malaise, headache, slight fever, weakness, grippy sensations, vague pains, insomnia, anorexia, nausea, loss of weight; slight focal reactions, as increased cough, expectoration, râles, pleuritic and laryngeal pains, vesical irritability, suppurative and other inflammatory signs; marked tenderness, pain, redness and swelling at the site of injection, are most important in therapeutic inoculations, and indicate that, for the time at least, the patient's tolerance to tuberculin has been reached; a further inoculation at this time may spell disaster. High fever, prostration, marked focal inflammatory signs and prolongation of systemic reactive phenomena mean that the patient has received an overdose of tuberculin and that treatment must be indefinitely suspended. Dogmatic instructions to increase progressively the dose every

three, five, or seven days, without due regard to the physiological effects, are criminal. No man should ever employ tuberculin who is ignorant of its physiological action. This action may escape notice, except by the skilled observer. The phenomena which do occur often demand keen discrimination as to the size and interval of subsequent dosage. The patient should have his temperature, pulse and respirations recorded at three-hour intervals for two or three days before and throughout the course of treatment. His weight should also be noted weekly. If febrile he must remain quietly in bed. In general the initial dose should be quite small in accordance with the directions given above for the particular tuberculin concerned. If no reaction whatever be produced, the dose may be doubled in three or four days. If there be slight evidence, general or focal, but nothing definite, the same sized dose should be repeated. If, however, a slight reaction occur, the next inoculation must not be given until all traces of the reaction have disappeared for three or four days, and then not more than half of the previous dose should be given. If, after any inoculation, a severe reaction be precipitated, no further inoculation shall be given for two or three weeks after the patient's condition has returned to normal, and the dose then must be not more than a fourth or a half of

the previous intolerant inoculation. Again the dosage ascends the scale, and this time will probably pass beyond the size of the former intolerant injection without harmful effect. Occasionally, a patient will be encountered exhibiting *tuberculin hypersusceptibility*. This does not refer to the ordinary response to a full-sized dose of tuberculin, but such a state as is seen when, after the reduction of the dose to one-half, the reaction reappears, and after a week or ten days with a further reduction to one-tenth, an even greater reaction occurs. This supersensitiveness to tuberculin can be overcome and immunization resumed by suspending all inoculations for three or four weeks, then beginning far down the scale of dosage; that is, one-thousandth of the former inoculation.

Experience has taught that most patients first show reactions to doses of tenths and hundredths of a milligramme. Consequently, when doses of these or larger sizes are administered, they should be spaced by a week or ten days instead of three or five days as is the flexible rule when administering the early small doses.

An all-important thought for the tuberculin therapist to bear constantly in mind is that in the average case it will require six to eight months to reach large immunizing doses; if the patient be hypersensitive the

time must be extended to a year or more.¹ Any attempt to "push the treatment," by shortening the intervals or carelessly increasing the size of the doses, will result in failure.

Limitations and Contra-indications of Tuberculin Therapy.—Two facts must be emphatically and indelibly impressed in the mind of the tuberculin therapist. They are: First, that tuberculin acts simply as an accessory to nature, and, second, that tolerance, even to large doses of tuberculin, does not necessarily confer immunity against tuberculosis. If these points are fully realized, tuberculin, in capable hands, will measure up to expectations and be accorded its rightful place in modern therapeutics.

Success in tuberculin therapy is directly proportionate to the degree of dosage attainable without producing deleterious reactions or intolerance. In the average case this is a procedure of months' or perhaps years' duration, in the event of the necessity of intermittent administration. Obviously, little can be expected from a short course of tuberculin inoculations with a maximum tolerant dose of only a fraction of a milligramme.

After the patient has been successfully carried

¹ Tuberculinum Purum, "T. P.," is an exception to this doctrine, since the patient may receive the entire series of inoculations in the course of three or four months, due to the lesser toxicity of this preparation, making it possible to ascend the scale of doses rapidly.

through a course of treatment and has reached large doses, these must not be continued indefinitely, lest the patient become over-stimulated and thrust into the "negative phase," hence no longer capable of the production of specific antibodies. It were better to cease inoculations for several months, when, if indications arise, a new course of treatment may be instituted.

As a general rule, tuberculin therapy is contra-indicated in acute, diffuse and chronic affections with acute exacerbations. It is far more applicable to so-called surgical or localized tuberculosis than to the pulmonary form of the disease. In short, it may be stated that tuberculin should not be employed in phthisis pulmonalis except in the afebrile or mildly febrile cases. It is definitely contra-indicated when a remediable surgical condition, as caries, sequestrum, tuberculous kidney, etc., exists, until after operative intervention. The usual acute or advanced case of pulmonary involvement should be invariably treated by rest in conjunction with the well-known climatic, dietetic, and hygienic antituberculosis measures. Subsequently, if there be no contra-indication and the patient is not progressing satisfactorily, tuberculin should supplement the treatment. Tuberculin therapy is decidedly contra-indicated in debilitated or wasted individuals or patients exhibiting very active lesions or extensive complications, characterized by considerable and in-

creasing fever, although slight dyspnoea, increased respirations, accelerated pulse-rate and even hæmoptysic tendencies, with or without evidences of dry pleuritis, are not necessarily contra-indications. Extreme caution must be exercised in practicing tuberculin inoculations on ambulatory patients, and particularly is this so if they are febrile, even though slightly and only occasionally. Menstruation and the supervention of an acute infection as a "cold," tonsillitis, bronchitis, etc., are definite indications for the temporary suspension of bacterial inoculations.

A matter of some importance in tuberculin therapy is the prevalence of *mixed infection* in tuberculous processes, either pulmonary or surgical. The advent of the secondary invading bacterium is characterized by an increased and typical irregularity of temperature. Under such circumstances culture and reculture of the suppuration from time to time and the preparation and administration of autogenous bacterins, preceding or alternating with tuberculin, will produce results not obtainable with tuberculin alone.

Indications and Results of Tuberculin Therapy in Tuberculosis.—Tuberculin is a powerful therapeutic agent in all localized, subacute and chronic forms of tuberculosis, not excluding the pulmonary type, provided the patient is not markedly asthenic or febrile. The immediate clinical effects of tuberculin

inoculations are oftentimes distinctive and impressive. Briefly summarized they are: (1) *local*, manifested by improvement in healing tendencies at a former operative site, disappearance of inflammatory signs, of cough, expectoration and tubercle bacilli from the sputum, although the last is very persistent and may never take place; (2) *general*, comprising improvement in appetite, digestion, strength, weight, disappearance of fever, etc., and (3) *mental*, that is, general morale.

The various forms of tuberculosis in which tuberculin has proved useful, if not curative, comprise pulmonary, genito-urinary, bones and joints, intestinal, peritoneal, laryngeal, ocular, mastoidal and lymph-nodal.

In most sanatoria, tuberculin is routinely employed in selected cases, namely, afebrile or mildly febrile patients, when an incipient infection may be aborted or the course of a chronic *pulmonary* process favorably influenced. In 1907, Trudeau stated that during the past 15 years the post-discharge mortality of patients from the Saranac Sanitarium was 18 to 25 per cent. less with tuberculin-treated than non-treated cases. Indeed this deduction is reasonable in view of the fact that tuberculin cautiously and competently administered is the best expectorant obtainable. Recently, *tuberculinum purum*, "T. P.," has been extolled in the treatment of this form of the disease (Fig. 18).

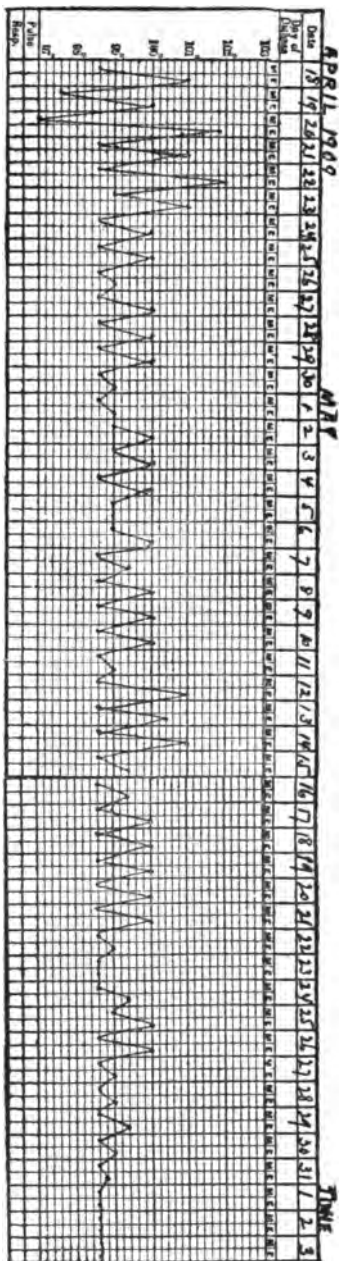


FIG. 18.—Chronio pulmonary tuberculosis. Treated by tuberculin inoculations twice weekly until 100 milligrammes were administered. (Courtesy of Dr. Frederick A. Dahl and Wm. Wood & Company.)

The forms of genito-urinary tuberculosis amenable to tuberculin include *bacillary tuberculous nephritis*, where both kidneys are functionally sufficient, but the primary or more affected kidney is indeterminable; bilateral, functionally insufficient, hence *inoperable tuberculous kidneys*; secondary *ureteritis* and *cystitis* following nephrectomy for tuberculosis and primary or secondary *epididymitis*, *prostatitis*, *seminal vesiculitis* and *salpingitis*. Exact discrimination must be exercised in the latter conditions as to whether the tuberculin syringe shall precede or follow the scalpel.

Perhaps the best results in tuberculin immunization have occurred in the treatment of *chronic osteitis* and *arthritis*. Frequently there is a superimposed mixed infection in these cases, calling for the preliminary or alternative employment of autogenous bacterins; repeated cultures become necessary at least biweekly, to identify the changeable pyogenic bacteria for preparation of the correct bacterin. We have treated many such cases of knee, spinal, and hip-joint disease with excellent results (Fig. 19). In acute osteomyelitis and arthritis, tuberculin therapy should be condemned.

Intestinal and *peritoneal* tuberculosis offers little promise for tuberculin. We have experienced remarkable results using tuberculin following exploratory laparotomy revealing extensive nodular tuber-

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culous peritonitis, and we have seen equally bad cases recover without tuberculin after simple laparotomy.

Tuberculous laryngitis is nearly always associated with pulmonary involvement and tuberculin has attained but indifferent success, although a few observers have reported excellent results. It would appear that the association of local treatment is a most important part of the management of this affection.

Ocular tuberculosis includes *chronic conjunctivitis*, *phlyctænar and interstitial keratitis*, *iritis*, *iridocyclitis* and *retinochoroiditis*, and seems to be a field wherein the results of tuberculin therapy have been most happy. Remarkable results have been obtained with "B. E." and to a less extent with "T. R." Old tuberculin seems to be definitely contra-indicated in all ocular affections.

Chronic mastoiditis and *otitis media*, at times, have responded brilliantly to tuberculin immunization. Again, when the inoculations have been without effect, operative intervention has revealed carious bone.

Tuberculin therapy in *chronic cervical lymphadenitis*, in the absence of indicated lymphadectomy, that is, when the nodes have undergone marked caseation or liquefactive necrosis, and particularly following the removal of diseased tonsils and adenoids, has enjoyed considerable success and should be employed in the future more extensively than has been the cus-

tom in the past. The patient's general health improves immediately, rapidly and markedly, and if old sinuses are present they close promptly. In our experience, equally good results have been obtained from "T. R.," "B. E.," "O. T." and "T. P." The results of treatment by tuberculin in this form of tuberculosis show that 75 per cent. are markedly improved, and that 40 per cent. of these will be cured, while 85 per cent. will demonstrate only slight improvement, and 10 to 25 per cent. must be counted as failures. The best results will be observed in children under the age of six.

XVII

PHAGOCYTOSIS

By phagocytosis is meant a property of the leucocytes whereby they take up into their substance foreign particles, such as bacteria (see Frontispiece), pigment, carbon granules, etc., thus removing them from the circulation. This power belongs pre-eminently to the polymorphonuclear neutrophiles, though phagocytosis by lymphocytes is occasionally observed. This phenomenon was discovered by Metchnikoff, who regarded it as the principal if not the sole means at the disposal of the body to rid itself of disease-producing germs. According to his teaching, phagocytosis included the taking up, the killing, and digestion of *living* bacteria, as well as absorption of dead bacteria and inert materials. Later studies of this phenomenon have led to modification of this comprehensive view by the majority of observers. It has, for example, been pointed out that the absorption of live bacteria by the leucocytes does not necessarily lead to their destruction, and the pus of gonorrhœa needs only to be cited as evidence. In gonorrhœa the great majority of the gonococci lie within the leucocytes and, far from undergoing destruction, rather tend to multiplication, at the same time retaining their highly infectious nature. Experiments with other

varieties of bacteria have shown that they also may retain their vitality after being taken up by the leucocytes. On the other hand, it has been shown that *dead* bacteria are taken up and digested by the leucocytes, and that insoluble foreign particles, such as carbon, pigment, etc., are absorbed and deposited in parts of the body where they will do no harm. It would appear, therefore, that this power of the leucocytes, known as phagocytosis, applies to the taking up of all foreign particles, irrespective of whether they are living or dead bacteria, pigment, etc., thus removing them from the circulation, but in itself has little or nothing to do with destruction of the life of bacteria. Phagocytic action is to be distinguished from the bactericidal action of the leucocytes, which depends upon separated soluble substances and does not take place within the leucocytes themselves.

Phagocytosis, therefore, is more or less a passive endeavor to remove foreign particles from the circulation, independently of whether they are living or dead, and in this way is to be regarded as one of the defensive forces of the organism against disease. The killing and destruction of living bacteria and neutralization of their toxins are carried out by means of bacteriolysins, opsonins, agglutinins, and antitoxins, in the soft tissues of the body, processes that are quite distinct from phagocytosis.

XVIII

RECOVERY FROM BACTERIAL INFECTIONS

IN the combat waged against disease, the body is equipped with natural defences that are called into play by the entrance of bacteria. In addition, each particular form of infection excites the production of antibodies that are specific for the disease in question. By means of these non-specific and specific defences, *spontaneous or natural* recovery is brought about.

In the case of the bacteria that produce their deleterious effects by means of separated soluble toxins, for example the diphtheria and tetanus bacilli, the body defences take the form of specific antitoxic substances, which, by neutralizing the toxins, cause a secondary destruction of the infecting organisms. Where the invading bacteria act by means of endotoxins that are inseparably bound up within their protoplasm, the body acts directly against the microorganisms themselves through various soluble bactericidal substances in the blood-fluid. After the bacteria have been killed, their bodies are taken up by the leucocytes (phagocytosis), and their endotoxins neutralized by non-specific oxidizing substances in the leucocytes.

Experimental and clinical evidence leads to the belief that there are in the blood two types of bac-

tericidal substances, (a) *leucocytic bacteriocidin*, (b) *humoral bacteriocidin*, and that certain varieties of bacteria are destroyed by the one, and other varieties by the other. These two types of bactericidal substances are found in the fluid part of uncoagulated blood, that is, in the plasma. If, however, the blood is allowed to coagulate, or is defibrinated, only the *humoral* substance is found in the serum. From this it is concluded that one bactericidal substance is derived from the leucocytes, and is probably identical with fibrinogen, while the serum or humoral bacteriocidin does not come from the leucocytes.

It has been found that streptococci and pneumococci are killed only by the blood-plasma, i.e., by the leucocytic substances; these organisms multiply in serum alone. On the other hand, typhoid bacilli are destroyed by serum or humoral bacteriocidins. In this way is explained the clinical fact that in streptococcic and allied infections there is a marked leucocytosis, while in typhoid fever the leucocyte count remains normal.

It has further been found that the humoral bactericidal substances lose their activity by heating the serum to 55° C., but that they can be reactivated by the addition of fresh normal serum; in other words, their action depends on the presence of complement. On the other hand, the leucocytic substances are not inacti-

vated by heating to 55° C., and are not dependent upon the presence of complement for their action. It is probable that the humoral bactericidal substances are specific, each one acting against a certain microörganism, while the leucocytic substance is non-specific.

Among the natural defences of the body also are substances in the blood known as *opsonins*, which have the property of preparing bacteria for phagocytosis by the leucocytes. Whether these are distinct soluble substances or only a property of the serum is not determined; it is quite likely opsonins are a modified form of the bactericidal substances in the blood-fluid.

From the foregoing discussion and from previous chapters, we see that the principal defences of the body against the deleterious effects of bacterial infection include antitoxins, agglutinins, bacteriocidins (bacteriolysins), opsonins and phagocytosis, each of these playing a distinct part in the spontaneous recovery of the organism from disease. By a knowledge of the way in which different varieties of bacteria produce their deleterious effects, and the resources present in the body for meeting the attack of the different species, we are enabled to assist nature in combating different forms of infection by *artificial stimulation* of these natural resources. This is brought about by the various general therapeutic measures, and in a *specific* manner by *bacterin therapy*.

XIX

BACTERIAL INOCULATION

PRINCIPLES UNDERLYING INOCULATION THERAPY—PREPARATION OF BACTERINS—AUTOGENOUS VERSUS HETEROGENEOUS PREPARATIONS—CLINICAL SYMPTOMS VERSUS OPSONIC INDEX IN CONTROL OF TREATMENT

Principles Underlying Therapeutic Inoculation.

—It has been elsewhere stated that bacterial inoculations operate by stimulation of tissue cells, after subcutaneous, intramuscular, or intravenous injection, to the production of specific antibodies, be they named agglutinins, bacteriolysins, opsonins, or what not. From their source, they are taken up by the lymph and blood-serum and distributed throughout the body. In the morbid process their action is to sensitize the invading bacterin, whereby they become more readily devoured by certain leucocytes, hence phagocytosis is promoted, or the antibodies by virtue of their lytic properties attack their specific bacteria, resulting in their disintegration (bacteriolysins), and tissue repair is favorably influenced.

A patient smitten by an acute infection is the victim of an inoculation of living bacteria (antigen). If his resistance be poor and the virulence of the invading microbe be great, he is prone to die; if his resistance be par or better, and the bacterium relatively

avirulent or the dose small, recovery is to be expected; on the other hand, although his vital resistance be exceptionally good, a severe virulent infection will probably result fatally. In any case it is obvious that the size of the living bacterial inoculation is too large—also more potent than the commonly employed maximum therapeutic inoculation of dead bacteria—for the welfare of the patient and he is temporarily thrust into the so-called “negative phase.” Therapeutically, therefore, bacterin therapy is indicated only in chronic or possibly also subacute localized infections. It is impossible to conceive by any stretch of the imagination the rationale or at least the necessity of their employment in acute or diffused infections in which the human organism is already overloaded with the products of a living antigen, or indeed its defences actually demolished and its system overwhelmed by the invading microbes; namely, such states as bacteræmia or septicæmia. Indeed, bacterial inoculations in such conditions not only add insult to injury but may be the determining forces for death instead of prolonged recovery. Administered in acute localized affections, as a rule unwisely perhaps, and certainly so unless the indications of the opsonic index are followed, either of two possible results may be expected. If the bodily resources are barely able to cope with the situation, and the local infection be very

active, additional injection of antigen will turn the tide of battle against the individual and an otherwise acute course be converted into a chronic or vital issue. On the other hand, if the individual's defences are great and his infection slight, an inoculation of bacterin may possibly increase his specific antibodies, thereby enabling him to abort or at least shorten the attack of his local infection. In the latter case, the probability is that the patient if left to himself will readily conquer his infection in due time. This discrimination, in view of the danger involved, between leaving Nature to herself and intervention by bacterin therapy, can be made only by recourse to determinations of the opsonic index. As a rule, these acutely localized, particularly if severe, and bacteraemic conditions constitute realms for the wiser and more logical practice of serum therapy or passive immunization.

Consensus of opinion generally supports the belief that the bacteria best adapted to bacterin therapy or active immunization are those containing the poisonous substances within themselves, the so-called "endotoxins," typified by the staphylococci and streptococci. Such bacteria do not liberate their endotoxins until disintegration or death of their organism occurs. On the other hand, diseases caused by the "exoteric" or toxin-producing microorganisms, typified by diph-

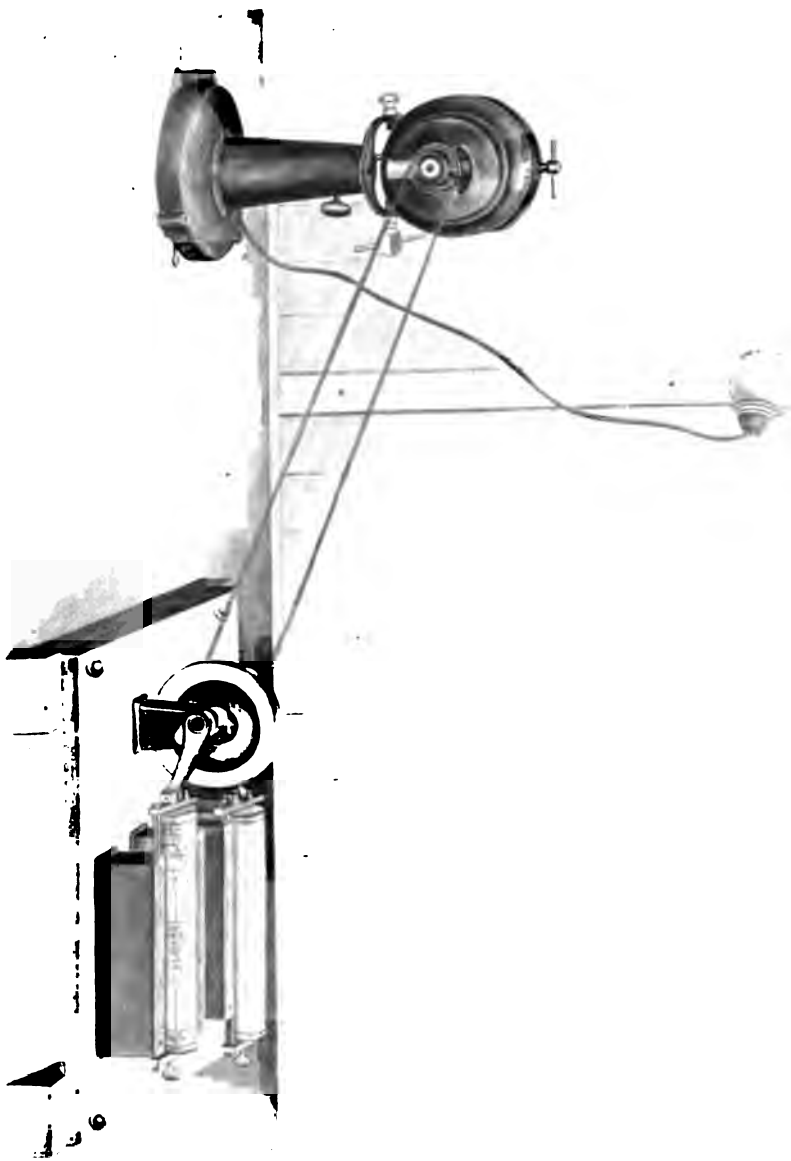


FIG. 20.—Electrical mechanical shaker. Fitted with rubber mountings to minimize vibrations. Bottles containing bacterial suspension are diagrammatically represented packed in cotton in specially devised brass cylinders, preparatory to shaking.

theria and tetanus, are more appropriately treated by immune sera or antitoxins.

Preparation of Bacterins.—The earliest, simplest and commonest method of bacterin preparation consists in washing off the appropriate media twenty-four-hour—with certain bacteria the time is much longer—pure cultures of the bacterium, using a platinum spatula. The growth is suspended in normal saline solution, containing, if desired, 0.25 to 0.5 per cent. phenol. After shaking for one-half hour in a mechanical shaker (Fig. 20), the suspension is standardized to contain from 100,000,000 to 1,000,000,000 bacteria per cubic centimetre. This is best accomplished by the hæmacytometer method, first employed by one of the authors (Thomas) in 1906. The bacterial suspension is drawn up in an ordinary red blood-cell counting pipette to the mark 0.5, followed by freshly filtered carbol-thionin, Leishman's or Jenner's stain, sufficient to stain the bacteria. After mild agitation for two or three minutes the bulb is filled to the 101 mark, and after thorough dissemination of the bacteria a drop of the pipette content is placed on the hæmacytometer stage and the microorganisms are counted precisely as are red blood-cells, determining the number per cubic centimetre. It is advisable to use a cover-slip not over 0.18 mm. thick; otherwise there may be interference with the oil-im-

mersion objective. Recently, Glynn, Rees, Powell and Cox have recommended a special counting-chamber 0.02 mm. deep, instead of the common 0.1 mm. depth, with which a heavier, 0.18 mm. thick, cover-slip, suitable for all achromatic oil-immersion lenses, even with a free working distance as low as 0.09 mm., can be used. It is claimed that with the shallower chamber the bacteria settle to the bottom in fifteen minutes, while with the deeper they are still in motion after one-half hour, therefore, with the special hæmocytometer they are more easily enumerated for focal reasons, their definition is clearer cut and the heavier cover-slip is more durable. Any hæmocytometer method is more accurate than Wright's method, which underestimates the number of bacteria in suspension and possesses an average and maximum error three times as great as the hæmocytometer.

Wright's method consists in mixing a unit volume of the bacterial suspension with an equal volume of normal blood known to contain approximately 5,000,000 erythrocytes to the cubic millimetre or 5,000,000,000 to the cubic centimetre. This is diluted by the addition of six or seven volumes of 0.15 per cent. sodium citrate solution to prevent clotting. After thorough mixing, fairly thick smears are made on glass slides and stained preparatory to the count. Fields throughout the smear are counted for the sake

of accuracy and the proportionate number of bacteria to the red blood-cells counted, determined as follows: If in fields containing 1000 red blood-cells 500 bacteria are counted we have the proportion

$$1000 : 500 :: 5,000,000,000 : x.$$

$$x = 2,500,000,000, \text{ or the number of bacteria per c.c. of suspension.}$$

This suspension is obviously too concentrated and may be conveniently diluted as follows: If it is desired to prepare the bacterin in 10 c.c. containers, so that each cubic centimetre shall contain 400,000,000 bacteria, we have the formula

$$\frac{400,000,000 \times 10}{2,500,000,000} = x = 1.6 \text{ c.c.}$$

Therefore to 1.6 c.c. of the original suspension must be added 8.4 c.c. of the diluting fluid, phenol-salt solution. The bacterin is then sterilized by submersion in a water-bath for one-half to one hour at the thermic death-point of the particular bacterium, usually about 60° C. After removal from the bath, a drop of the bacterin is cultured for a day or two in order to test its sterility before employment therapeutically. Other methods of sterilization occasionally utilized include chemical, cold and maceration by prolonged shaking.

Preparation of Bacterins by Autolysis.—Certain investigators have attempted to improve bacterins and enhance their immunizing properties by subject-

ing the bacteria to a process of autolysis, on the assumption that the autolysis contains toxic or antipsonic substances, and that the antigenic part of the bacterin depends upon the bacteria themselves minus their extractives. This disintegration of the bacteria is accomplished by treating them with salt solution, alcohol, ether, chloroform, or xylol, followed by filtration and suspension of the residue in normal saline.

Serobacterins.—Recently, advantage has been taken of Besredka's assertion, founded upon the demonstration of Ehrlich and Morgenroth, that inoculations with sensitized bacteria supersede in effectiveness, rapidity of action and harmlessness ordinary bacterins or bacterial vaccines. Such sensitized bacterins or "serobacterins" are prepared by treating bacteria with their specific immune serum, by which process the bacteria become inseparably joined with their antibodies, thereby in a state, immediately on injection, prepared to be acted upon by the complement of the patient's blood. Thus the usual delay of a week or more, required for the patient to form his own antibodies—incidentally also the negative phase and local injectional reaction—using the ordinary bacterins, is avoided, and immunity is secured rapidly and intensively in twenty-four to forty-eight hours. Finally, by virtue of the absence of "negative phases," the inoculations may be repeated every day or two, thereby

markedly shortening the course of treatment. Should sensitized bacterins fulfil their promise, they will mark the greatest advance thus far in the history of bacterin therapy.

Bacterins, like tuberculins, are best stored in a dry, cool, dark place. As a matter of convenience they are put up in hermetically sealed ampoules or small



FIG. 21.—Various forms of containers for storage of bacterins.

bottles or vials fitted with ground glass stoppers or rubber caps (Fig. 21). The standardized strength of the bacterin, that is, the number of bacteria per cubic centimetre, also its date of preparation, should be inscribed on the container. The preservative, phenol, usually added to the diluting fluid, insures against contamination or bacterial growth in the preparation. It should be remembered that the bacterin may ap-

pear perfectly clear after a certain time owing to digestion of the previously discernible microorganisms. On the other hand, it often happens that the bacterin becomes clouded or a precipitate forms, due to the chemical action of the contained phenol. Such bacterins should be immediately discarded. As a rule, bacterial suspensions in trikresol-saline solution will keep for months. It is alleged that typhoid bacterin becomes inert after three months.

Autogenous versus Stock Bacterins.—By an autogenous or personal bacterin is meant one that is prepared from the particular bacterium or strain of bacterium cultured from the infected patient. A stock or laboratory bacterin is understood to mean a preparation in which the bacterium has been isolated from another patient, who has suffered from a similar infection. In view of the diversity of the strains of many bacteria, notably the streptococcus, colon bacillus, etc., also of the fact that not a few diseases are caused by different bacteria, namely, abscess, acne, etc., it is natural that the preferable and most scientific procedure is to employ an autogenous bacterin whenever possible. The authors have often successfully treated cases with an autogenous preparation after stock bacterins have resulted in failure. Not infrequently it becomes expedient to use a stock preparation, at least while the autogenous bacterin is being

prepared. Under such circumstances, notably in gonorrhœal and tuberculous affections, it is imperative that the infecting germ be accurately determined. Moreover, the bacterin should be polyvalent, that is, composed of as many different strains of the bacterium as may be obtainable, and in view of the reports of Besredka, Broughton-Alcock, Ardin-Deltiel, Nègre, Raynaud, Gordon, Boinet, Cruveillier and others, it would appear advisable to employ so-called sensitized bacterins.

Clinical Symptoms versus Opsonic Index in Control of Treatment.—Regulation of the size of doses of bacterins and the spacing of the intervals between inoculations is possible by observations of the clinical symptoms, subjective and objective, and by determinations of the opsonic index. Both methods involve special knowledge and are not to be undertaken lightly. The former entails the closest kind of observation respecting local, focal and general responses (see Chapters XVI and XXI). Opsonins are not the only antibodies formed after inoculation of an animal, hence the opsonic index, theoretically, cannot measure the full degree of immunity.¹ Practically, the opsonic index in the majority of cases runs par-

¹ There is no such thing as absolute immunity, however, a fact pointed out by Pasteur years ago, for an animal will contract disease, irrespective of the degree of immunization, provided the dose of infective material is sufficiently large and virulent.

allel with the clinical symptomatology, and although in many cases it is unnecessary to employ the index as a guide, there are many cases in which dependence upon it is absolutely essential to attain the greatest success. To ignore the index absolutely in all cases will invite disaster. The authors feel, after an experience of many years, that the most brilliant results—often in the most difficult cases—have attended bacterin therapy, wherein the opsonic index was associated in the management of the case; although they are inclined to attach primary importance to the clinical symptomatology, properly interpreted, and to relegate the index to second place. For instance, in the treatment of acne or recurrent furunculosis, we are not infrequently at a loss, after the disappearance of the present lesions, to say whether or not the process of immunization has been carried far enough to insure no recurrence, or in certain other affections, characterized by a high fluctuating temperature and toxæmia, or in deep-seated lesions, such as pyelitis, pyelonephritis, cystitis, etc., the value of the index in controlling dosage and, in the case of mixed infections, in selecting the needed bacterin, is not inconsiderable. The treatment of this class of conditions lies without the sphere of the general practitioner and he would do well to refer such cases to those more particularly versed in the application of bacterial in-

oculations. On the other hand, physicians generally, at the present day, owing largely to the energy of pharmaceutical firms marketing stock bacterins, employ bacterins controlled or miscontrolled by the clinical symptoms in preference to the opsonic index.

It is utterly out of the question for the average general practitioner to master the bacteriology and laboratory technic required for the reliable determination of the opsonic index, and experience has already demonstrated that in the majority of patients thus far subjected to bacterial immunization the clinical symptomatology has admirably sufficed to control the inoculations. Consequently, bacterin therapy, in order to enjoy the popularity which is its due, must be governed, within limitations, by the subjective and objective symptoms and signs, observing the former and avoiding the latter.

XX

THE OPSONIC INDEX

**DEFINITION OF OPSONINS AND THE OPSONIC INDEX—
TECHNIC OF DETERMINATION OF THE OPSONIC INDEX
—INTERPRETATION, VALUE AND LIMITATIONS OF THE
OPSONIC INDEX**

DENYS and Leclef, Leishman, Wright and Douglas and others have demonstrated that the real activity of phagocytes depends upon a specific substance—the exact chemical nature of which has been undetermined—existing in the serum of the blood. Irrespective of a certain amount of *spontaneous phagocytosis*, which has been shown may take place, Sir Almroth E. Wright, of St. Mary's Hospital, London, has definitely proved that the subcutaneous injection of dead bacteria is capable of the production of specific antibody, which taken up by the blood-serum markedly augments the ability of the phagocytizing leucocytes to devour invading pathogenic bacteria. To this specific substance, capable of preparing bacteria for ingestion, Wright assigned the name "opsonin," from the Greek verb ὀψώνω, I prepare food for. *Opsonins, therefore, are those specific substances or antibodies, not yet isolated, in the blood-serum, possessed of the property to sensitize or prepare bacteria for phagocytosis.* Opsonins are bacteriotropins

and should not be confounded with bacteriolysins, another type of antibody. Moreover, it has been shown that opsonins are divisible into thermolabile (the normal opsonin of Wright) and thermostable (immune opsonin). The former are readily destroyed at a temperature of 56° C., the latter retain their bacteriotropic property in spite of this degree of heat.

By virtue of the assiduous labors of Wright and his co-workers in evolving a technic (determination of the opsonic index) to measure phagocytosis (degree of immunization), therapy by bacterial inoculations was revived and popularized to an unprecedented extent and has led to the application of active immunization of far-reaching consequences, and it is for this reason that medicine owes Wright a perennial debt.

The opsonic index may be described as the measure of the ratio of the phagocytic activity of neutral or washed leucocytes in the patient's serum for given bacteria, as compared with those in a normal or control serum. Inasmuch as a neutral phagocyte will ingest the same number of bacteria, provided the two sera possess identical qualities, the normal or base line is arbitrarily taken as one. If, in the case of the patient's serum, it is found that 100 leucocytes contain 900 bacteria, while the same number of leucocytes treated with the control serum contain 1000 bacteria,

we have the proportion of $900 : 1000 :: x : 1$, in which x , the opsonic index, equals 0.9.

Technic of Determination of the Opsonic Index.

—The technic of the opsonic index is a rather intricate laboratory procedure, which, to be reliable or trustworthy at all, involves much practice in mastering the details as stipulated by Wright. It demands that the opsonist shall be a thorough bacteriologist as well as an experienced laboratory worker. Briefly, the procedure may be described as follows:

Material and apparatus required,

- (a) Pure culture of infective bacterium.
- (b) Specimen of serum of patient's blood.
- (c) Specimen of serum of normal or control blood.
- (d) Normal washed leucocytes.
- (e) Centrifuge, preferably electric.
- (f) Thermostat or opsonizer.
- (g) Centrifuge or special test-tubes.
- (h) Teat and capillary pipettes, glass tubing, slides, absolute alcohol, stains, etc.
- (i) Microscope.

A few drops of the patient's blood and of the normal or control blood are collected in two glass tubes drawn out on either end to capillary size (Fig. 22). The straight or distal end, free of blood, is sealed in a flame, and in a few minutes, after the tube cools, dur-

FIG. 23.



FIG. 24.



FIG. 23.—Showing the collection of blood in sodium citrate saline solution, in order to obtain washed leucocytes.

FIG. 24.—Blood after centrifugation in decalcifying medium, exhibiting the layer of "leucocytic cream" overlying the erythrocytes.



FIG. 25.—Electric centrifuge. This instrument is particularly well adapted for the preparation of washed leucocytes by virtue of the easy manner in which it loses speed when the current is broken, thereby avoiding disturbance of the layer of "leucocytic cream."

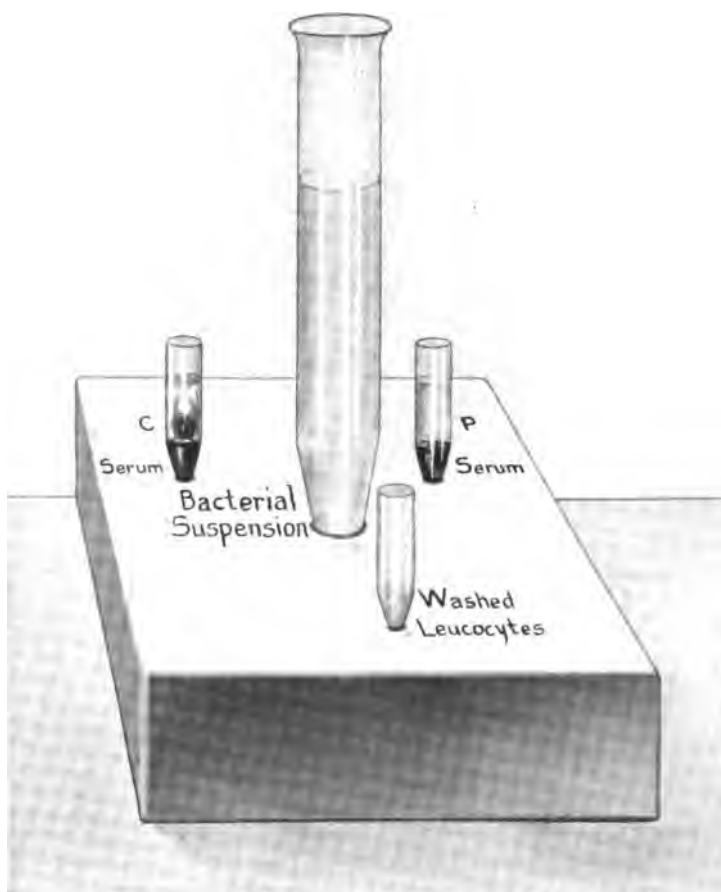


FIG. 26.—Various ingredients necessary for the determination of the opsonic index

ing which time the blood coagulates and is spontaneously drawn toward the sealed end, the curved or inlet end may also be sealed. The serum begins to lose its opsonic content in six hours and should not be utilized for index determination after this time. Ten

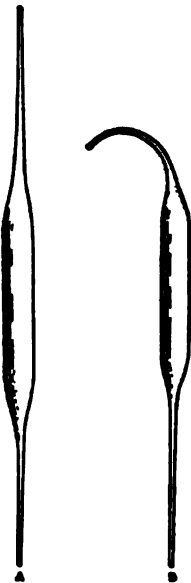


FIG. 22.—Capillary glass capsules for the collection of specimens of blood. A, simple straight; B, Wright's.

to fifteen drops of normal blood are then allowed to fall from the finger-tip into a test-tube of normal salt solution containing 1.5 per cent. sodium citrate (Fig. 23). The citrate will prevent the clotting of the blood, and the corpuscles are thoroughly washed by centrifugation two or three times in normal saline (Fig. 25), to render them neutral and absolutely free from serum. The white blood-corpuscles, being lighter than the red, will overlie the latter as a grayish layer of "leucocytic cream" (Fig. 24), whereupon they may be picked up by a pipette and transferred to another receptacle (Fig.

26). A six- to twenty-four-hour culture of the given bacterium is washed off in normal salt solution (Fig. 27), then centrifuged to throw down all bacterial clumps. The supernatant suspension should contain only individual bacteria in not too

great concentration. The best strength would appear to be that rendering a phagocytosis averaging about four bacteria pro leucocyte. Two opsonizing capillary pipettes, *P* (patient) and *C* (control), are prepared so that the lumina are about the diameter of a large sized hat-pin. A distance of one-half

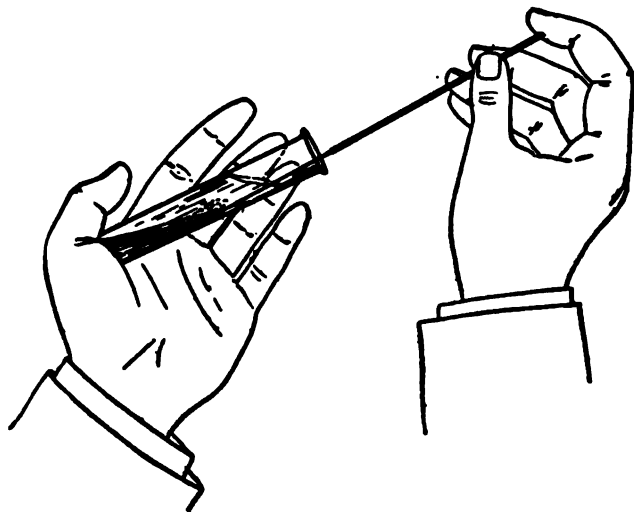


FIG. 27.—Washing the culture of the given bacterium from the culture medium, using the platinum loop, in order to obtain the bacterial suspension.

or three-quarters of an inch is marked off on the ends of these two pipettes (Fig. 28, I and II). By the use of a small rubber bulb, equal quantities of patient's serum, bacterial suspension and washed leucocytes, permitting a small bubble of air to separate each, are drawn up and mixed by skilful thumb and finger gymnastics, in the pipette designated *P*. In the same

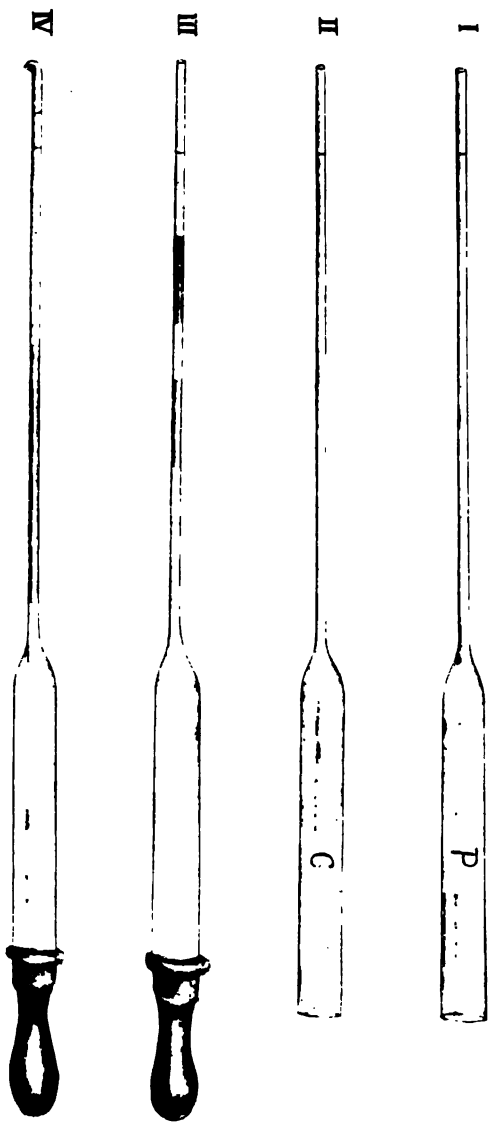


FIG. 28.—Opsonizing capillary pipettes. I, pipette accurately graduated and prepared for patient's serum. II, pipette accurately graduated and prepared for control serum. III, pipette containing equal quantities of serum, bacterial suspension and washed leucocytes, separated from each other by a bubble of air. IV, pipette with sealed tip after serum. Bacterial suspension and washed leucocytes have been thoroughly mixed, preparatory to incubation in opsonizer.

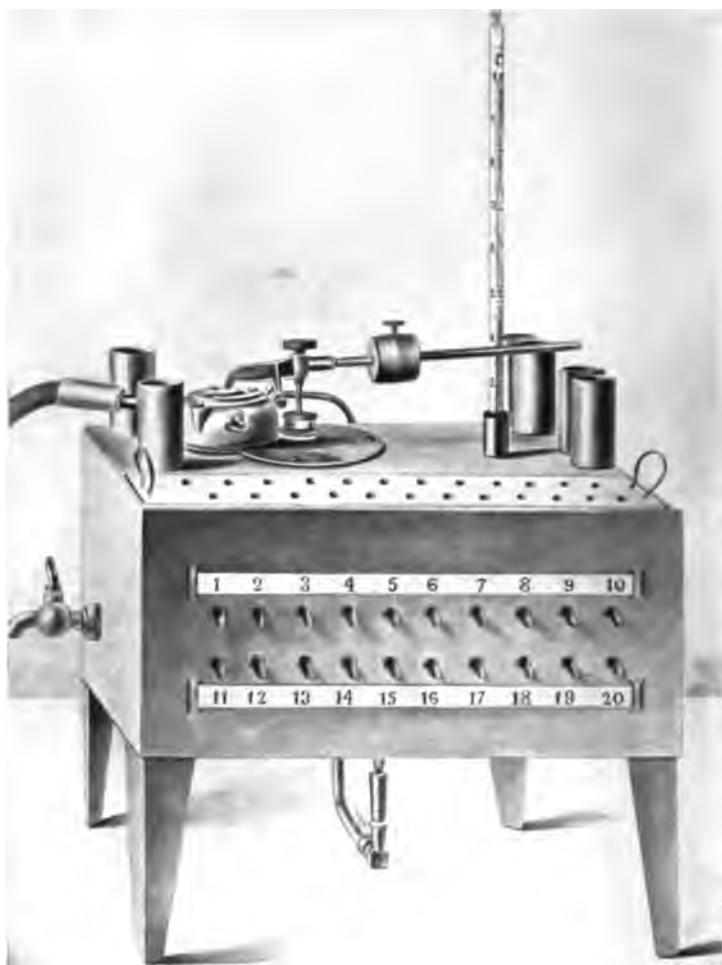


FIG. 29.—Opsonizer or thermostat. This incubator is equipped with an automatic heating device and permits of the ready manipulation of pipettes introduced through the side port-holes.

manner equal quantities of control serum, bacterial suspension and washed leucocytes are taken up and mixed in pipette *C* (Fig. 28, III and IV). The tips are sealed in a flame and the two pipettes incubated for a few minutes in a thermostat or opsonizer at 37° C. (Fig. 29). Care should be taken to agitate

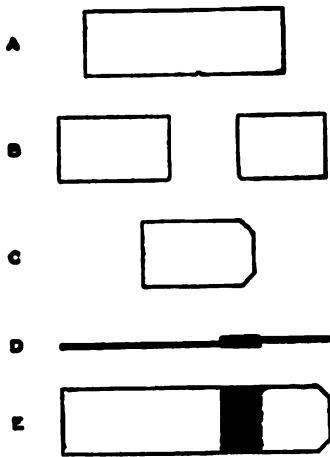


FIG. 30.—Illustrating the construction of Kuhnhardt's spreader. A shows a glass slide properly nicked by a file; B, the slide properly broken in a slightly concave line; C, the broken slide with corners clipped; D and E, the perfected spreader spliced to a second slide with adhesive plaster.

the contents every five minutes during incubation, otherwise the corpuscles will gravitate and a perfect admixture of serum, bacteria and phagocytes will not be obtained. At the conclusion usually of fifteen minutes, smears are made of the contents of the pipettes on cover-slips by the usual method or glass slides, employing Kuhnhardt's spreader (Figs. 30 and 31). After drying,

the smears are fixed in absolute alcohol and stained with freshly filtered carbolthionin or by the method of Homer Wright. In the determination of the tuberculo-opsonic index, the culture of tubercle bacilli is killed by fractional sterilization on three successive days at 100° C., then thoroughly ground

in an agate mortar and suspended in 0.85 per cent. solution of sodium chloride. After fixation of the smears either by ethyl or methyl alcohol or by heat, the usual staining method comprising carbol-fuchsin, nitric acid and methylene blue for tubercle bacilli is employed (see Frontispiece). The number of bac-

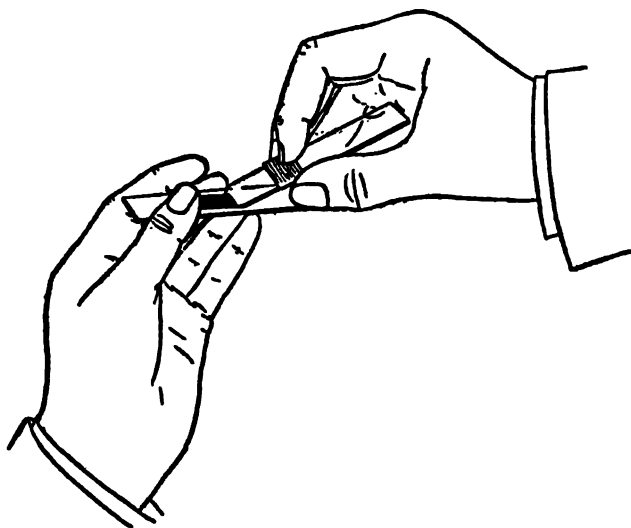


FIG. 31.—Kuhnhardt's spreader properly held at the right angle for the preparation of a satisfactory smear.

teria contained in no fewer than one hundred phagocytes should be counted and the index determined as noted above; or, in accordance with the method of Simon, the ratio of the number of phagocytizing leucocytes may be determined. The latter method is simpler, less exacting on vision and possibly just as reliable as the method of Wright.

Interpretation, Value and Limitations of the Opsonic Index.—Wright contends that the opsonic index, properly done and interpreted, is a reliable expression of an animal's resistance against infection. The effect of a bacterial inoculation, after a transitory drop in the opsonic index, is an increase in the degree of immunity of the inoculated animal against the disease caused by the given bacterium. The primary drop or so-called "negative phase," presumably due to the absorption of preformed opsonins by the injected bacteria or to the systemic effect incident to the cellular stimulation of the organism for the production of specific antibodies, may be of very short or prolonged duration, and if of any consequence is characterized by the clinical phenomena, general, focal and local, described in the following chapter. In a few hours, after a proper sized dose, the opsonic index rises in the scale. This ascent is termed the "flow" and may fail to reach the normal or soar well above it. Since the effect is but transient, the index will slowly fall and this descent is called the "backflow" or "ebb" or "negative phase," occasionally following an inoculation, if the indices are determined sufficiently often, the curve will exhibit a mild "initial rise" preceding the "ebb" or "negative phase," before the real "flow" or "positive phase" sets in

the "ebb" is less conspicuous and the "positive phase" becomes sustained. At this time the patient is said to be on the "high tide of immunity" and further inoculations are contra-indicated. This is observed in connection with prophylactic inoculations and where recovery is apparent. Wright's original rule was to take the opsonic index daily and be guided exclusively by its indications; at present, although the value of the index is not belittled, the clinical course of the case is utilized as an important guide in treatment. Inoculations are not repeated until positive phase symptoms are no longer apparent, or in doubtful cases determination of the index demonstrates a fall in opsonins. So long as there occurs any beneficial response whatever from a bacterial inoculation, its size, whether the "minimum effective" or the "medium or average," is not increased.

The "ideal curve" consists of a "negative phase" of twelve or twenty-four hours, followed by a "positive phase" lasting from three to fourteen days. This curve depends upon two factors, (1) the size of the dose and (2) the vital resistance of the patient. A small dose may have to be repeated frequently, possibly every day, owing to the short duration of the "positive phase," whereas a large dose may necessitate the suspension of any further inoculations on ac-

count of a prolonged "negative phase," or possibly the establishment of immunity and recovery. Failure

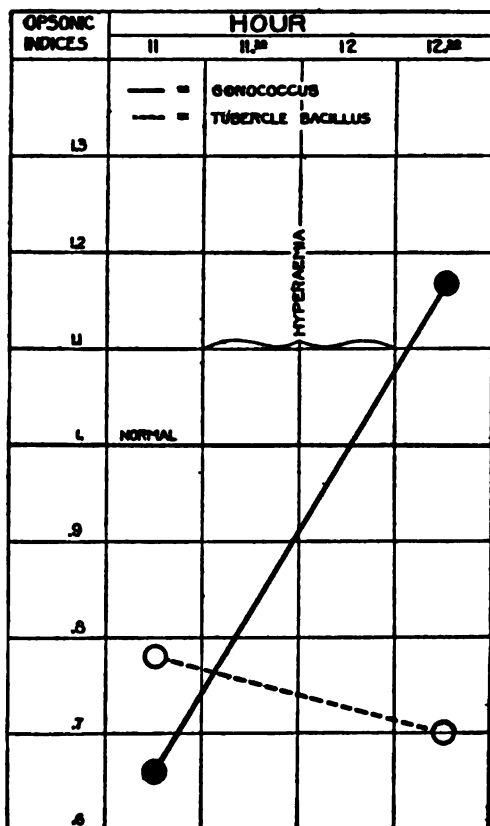


FIG. 33.—Case of gonorrhoeal arthritis of knee, showing manner of differentiation between the possible causes of synovitis or arthritis. The opsonic indices for the two suspected etiological organisms were determined before the application of passive hyperaemia to the infected joint. After intermittent application for one and a half hours the indices were again taken, with the result that the opsonic index for the gonococcus, the specific organism in this case, had risen considerably.

to evoke a rise in the opsonic index may be attributable to (1) too small dosage, (2) reinoculations during the negative phase, or (3) inoculation treatment

of patients, wherein bacterins are contra-indicated, namely, those already overwhelmed by their infection.

In addition to its value as a control of treatment in bacterin therapy, the opsonic index, in expert hands,

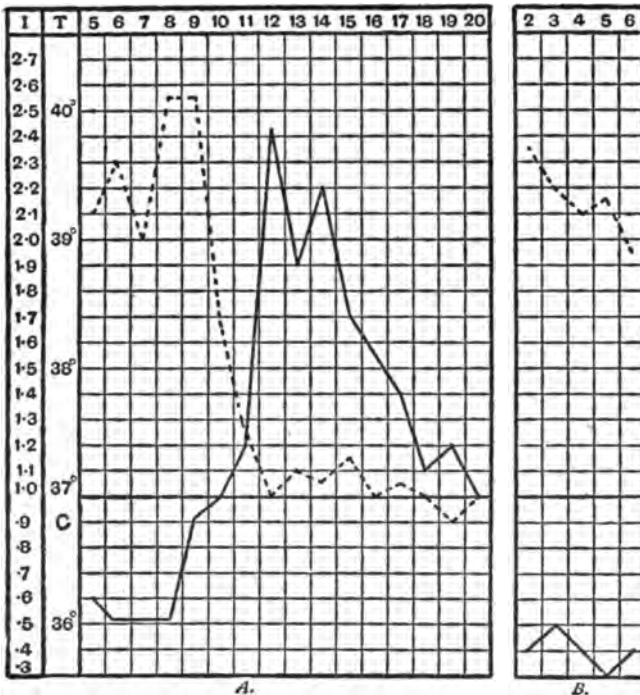


FIG. 34.—Pneumonia. *A*, recovery; *B*, death; —, curve of opsonins (I); ---, curve of temperature (T). Observe in the favorable case a rise in the opsonic index coincidently with the fall in temperature. (Giglioli and Stradotti.)

has been utilized both diagnostically and prognostically (Figs. 33 and 34). An important sphere of its utility may be in the determination of the degree of animal immunization and standardization of sera, and has been so employed by the authors. Its value in a

diagnostic and prognostic capacity would appear to be limited, (1) because other methods, as the cutaneous allergic reactions, are simpler if not more reliable, and (2) owing to a certain amount of error, even with

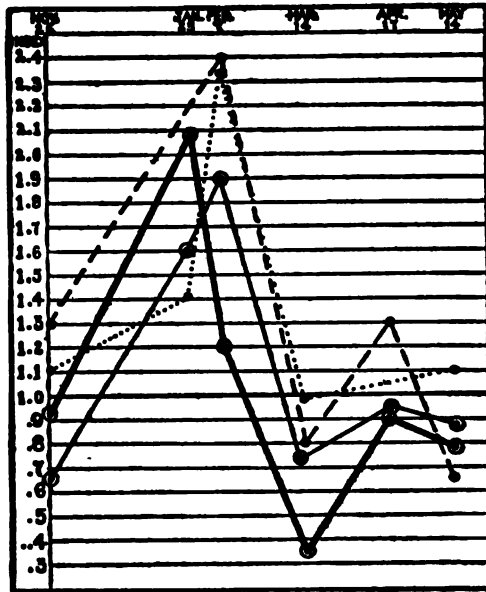


FIG. 35.—Showing effect of variable concentrations of bacterial suspension on determinations of opsonic index. —, bacterial suspension centrifuged for 10 minutes, then dilution of supernatant suspension so that 1 c.mm. contained approximately 10,000,000 bacteria., bacterial suspension centrifuged for 10 minutes. - - - - -, bacterial suspension centrifuged for 10 minutes and supernatant suspension diluted with 3 volumes of 0.85 per cent. saline solution. —, bacterial suspension standardised by McFarland's "nephelometer."

experienced workers, small variations may not be dependable, although it renders great service at times, in cases of mixed infection, enabling the immunologist to choose for inoculation the particular responsible

bacterium, or in the course of immunization to select from time to time the bacterin to which the index is low. It is generally admitted that the error due to technic alone, aside from the question of personal equation, amounts to one- to two-tenths. This variability is dependent particularly upon (1) the number of cells counted and (2) the concentration of the bacterial suspension (Fig. 85).

XXI

PRACTICAL APPLICATION OF BACTERIAL INOCULATION IN MEDICINE, PROPHYLACTICALLY AND THERAPEUTICALLY

GENERAL CONSIDERATIONS—INDUCED AUTO-INOCULATION—DURATION OF ACTIVE IMMUNITY—MODES AND TECHNIC OF ADMINISTRATION OF BACTERINS—DOSAGE—CONTRA-INDICATIONS, LIMITATIONS AND CAUSES OF FAILURE IN BACTERIN THERAPY—APPLICATION AND RESULTS OF BACTERIAL INOCULATIONS IN SPECIAL DISEASES—DISEASES OF THE SKIN AND SOFT PARTS—DISEASES OF THE GENITO-URINARY SYSTEM—DISEASES OF BONES AND JOINTS—DISEASES OF THE EYE, EAR, NOSE AND THROAT—DISEASES OF THE LUNGS—DISEASES OF THE ALIMENTARY SYSTEM—DISEASES OF THE CARDIOVASCULAR, LYMPHATIC AND NERVOUS SYSTEMS, ALSO OTHER ACUTE SPECIFIC FEVERS—MALIGNANT NEOPLASMATA—YEAST AND SOUR MILK

General Indications.—Wright has declared that, “we have in the power of increasing the antibacterial power of the blood by the agency of vaccines, and in our power of bringing the antibacterial agencies of the blood into operation in the focus of infection, beyond all comparison the most valuable assets of medicines.” It might be wise to modify this assertion to the effect that, *in the competent employment of bacterins we have one of the most valuable assets.* A very important fact ever to be remembered in the practice of medicine—and this includes particularly bacterin therapy—is never to discard or disregard old-established measures of proven worth, for a new idea,

no matter how attractive and possibly of superior value, for, by the skilful association of all, cure will be best promoted and facilitated. Wright's doctrine has become classical: "The medical man who has recourse to vaccine therapy ought to have familiar acquaintance with the microbes which affect the human body. He ought to appreciate the fact that vaccines owe their efficacy to the reaction they set up in the tissues, and not to any action exerted directly by the vaccine upon the invading microbe. He ought to have mastered the general principles of immunization. He ought to know in connection with each vaccine the minimum effective dose, *i.e.*, the dose which gives the minimum immunizing reaction without any intervening negative phase; and the medium or average dose, *i.e.*, the dose that gives, after a negative phase, a more powerful immunizing reaction. He ought to know the general conditions which affect the sensibility of the organism. He ought to understand how to adjust the dose to the requirements of the individual patient, and he ought to have a knowledge of the conditions which obtain in the focus of infection, and of the methods of circumventing the difficulties which are introduced by these conditions."

It is extremely improbable that bacterin therapy as practiced by the average general practitioner is destined to realize the full measure of its promise,

either because he lacks the required bacteriological facilities or is too busy to devote the necessary time and attention to trivial signs and symptoms. It seems not to be appreciated by physicians generally that bacterins are agents capable equally for good or evil. It cannot be too strongly emphasized that bacterin therapy is merely a valuable accessory to Nature in the art and science of healing and is not a "cure all." Carelessness, ignorance, incompetence or a desire on the part of the therapist to *push the treatment*, when beneficial results become apparent, will lead not only to failure, perhaps disaster, but at all times discredits a therapeutic measure of great value, and deprives the patient of his natural resources for recovery. Two solutions for the problem are offered the general practitioner contemplating bacterial inoculations: either to familiarize himself more with immunology, including bacteriology and laboratory methods, or to refer his patient to or coöperate with an immunologist, precisely as he is accustomed at times to consult an ophthalmologist.

Induced Auto-inoculation.—Therapeutic inoculation may assume one of two forms, (1) exogenous injection of bacterial suspensions, forming the main theme of this chapter, and (2) endogenous inoculation or auto-inoculation. The latter is a very common clinical phenomenon and may be *spontaneous* or *induced*.

Spontaneous auto-inoculation is frequently observed in a patient the victim of subacute or chronic phthisis pulmonalis, and is characterized by the irregular flares of temperature. If the doses of bacteria or their products, which at these times are cast off into the general circulation, are too great or repeated too often, the pa-

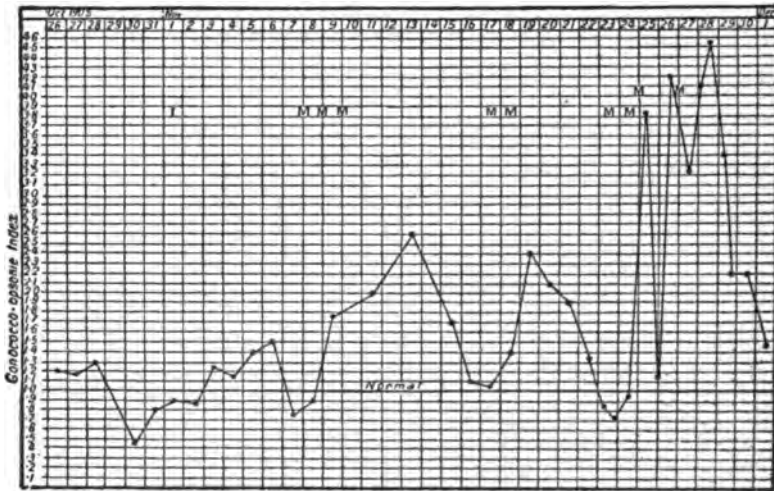


FIG. 36.—Case of gonococcal arthritis. Showing the effect of massage on the gonococco-opsoneic index. I=inoculation of 50,000,000 gonococci. M=massage. (Dr. J. Freeman.)

tient is thrust into a profound or permanent "negative phase" and death supervenes. If, on the other hand, the dose of re-infection be of the proper size and occurs opportunely, the patient becomes to a certain extent immunized and at least a temporary recovery takes place. Induced or artificial auto-inoculation is a most interesting problem and at times has

proved feasible in the treatment of various infections. It would appear advisable oftentimes when bacterin or vaccine therapy is contra-indicated. It consists in an endeavor to inoculate the patient from his own lesion by purely physical measures. These include massage, exercise, applications of heat, passive hyperæmia, etc.,

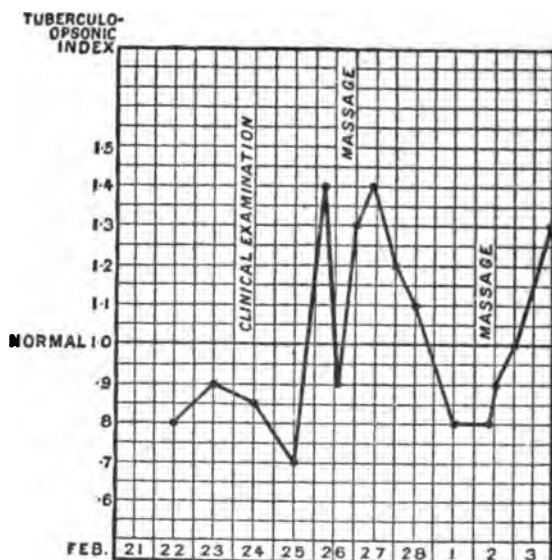


FIG. 37.—Case of tuberculous cervical lymphadenitis. Showing effects of massage on tuberculo-opsonic indices. (Wright.)

and explains wherein lies the diagnostic value of the opsonic index, for example, in gonorrhœal arthritis (Figs. 33 and 36), and in tuberculous cervical lymphadenitis (Fig. 37). The production of induced auto-inoculations may result in excessive dosage, as we have no means to gauge, at the moment, the size of

the dose cast into the circulation, and in view of the fact that the inoculation consists of living bacteria, not as in ordinary bacterin therapy of measured dead microörganisms, the method is attended by a certain amount of danger and should be utilized only by experienced hands.

Duration of Active Immunity.—In general it is admitted, and may be stated, that the greatest and most lasting immunity is produced by inoculations with living bacteria, then with dead bacteria and finally with the products of bacterial growth. This has been quite satisfactorily demonstrated by animal experimentation, and a common procedure is to begin the process of immunization with inoculations of dead bacteria, followed by the living microörganisms. Thus far, the profession has not seen fit, nor would patients submit to injections of living bacteria, although by resort to the technic of Williams and Webb—inoculations at first with a single living bacterium, then by cautious count progressively increasing the dosage—or supplementing inoculations of dead by living or attenuated bacteria, no danger should be incurred, and the day may not be far remote when such a practice in expert hands will be an unobjectionable and common procedure.

Immunity is never absolute, but the protection afforded by bacterial inoculations lasts indefinitely, fre-

quently for months or years. There is substantial evidence that prophylactic typhoid immunization protects the individual for at least three years. The duration of immunity may be determined by cutaneous allergic tests and by serological reactions, including the opsonic index.

Modes and Technic of Administration of Bacterins.—Bacterins have been administered subcutaneously, intravenously, orally and per rectum. The last three methods are not in general use, in spite of sporadic marvellous results, nor do they appeal strongly on immunological grounds, and consequently seem not destined to become popular. Subcutaneous administration is the procedure usually practiced. In the event of mixed infections, multiple bacterins of the respective pure bacterial cultures may be prepared for alternate or indicated inoculations, or a mixed bacterin proportionately compiled of the various invading microorganisms may be employed. The technic consists in sterilizing the skin with a pledget of cotton and alcohol overlying the insertion of the deltoid muscle or elsewhere on the body, as described on page 178. It is recommended whenever possible to inoculate on the distal side of the lesion, thereby giving the patient the immediate benefit of the antibody formation through the local lymph system in addition to the subsequent effect to be obtained through the blood-

serum. The syringe (Fig. 38) constructed entirely of glass or one having an asbestos piston is most serviceable, as it permits of ready cleansing and sterilization by boiling. When inoculations are given on a large scale, requiring frequent sterilization, the oil method of Wright is novel, convenient and effective. It consists in heating a quantity of olive oil over a gas flame. The oil will heat to 150° C. without boiling. This is one and a half times the temperature of boiling water and instantly sterilizes the syringe when drawn up and forced out after inoculation. From the ampoule after



FIG. 38.—All-glass hypodermic syringe. Desirable for bacterial inoculations because of ease of cleansing and surety of sterilisation.

filing or breaking off its neck or from the small bottles,—in the case of the rubber-capped one by plunging the needle through a drop or two of phenol, formalin or alcohol placed on the rubber,—the desired dose of bacterin is drawn up into the syringe. In the case of some vials with rubber stoppers it is expedient to withdraw partially the piston of the syringe before thrusting it through the cork, then to push it home and as it is slowly withdrawn again the desired quantity of bacterin readily follows the piston. If the skin is firmly grasped and lifted between the finger and thumb and

a perfectly sharp pointed fine needle is employed, the amount of pain occasioned by the puncture is extremely slight. The bacterin, if a large dose, should be slowly injected into the loose subcutaneous tissue to minimize pain. As the needle is withdrawn the site of the puncture should be compressed and the skin gently stroked a couple of times with the pledget of alcoholized cotton. No further dressing is necessary or desirable.

Dosage.—The size of the dose of bacterin must be cautiously gauged. The two fundamental considerations are (1) the nature of the particular infecting bacterium and (2) the condition or state of the patient. It should be thoroughly realized that not only bacteria in general but individual strains of the same bacterium differ greatly in virulence and, as a rule, the more virulent the microbe the smaller should be the inoculation. To determine this and the immunizing power of the bacterin, with the greatest precision, various clinical laboratory and blood tests are essential. Thus the dose of the more virulent bacteria, as the streptococcus, pneumococcus, gonococcus, colon bacillus, etc., should be considerably smaller, that is, one-half to one-fourth that of the less virulent microorganisms, as the staphylococcus. Again, a most important problem is the determination of the *minimal effective* and the *average and maximal* dose for each bacterium. In desper-

ately or acutely ill patients, or with patients in whom a beneficial response has been obtained with the minimal effective dose, this should not be exceeded. With chronic cases, presenting well-localized lesions, the average or maximal dose *may* be administered at the start, and subsequently increased. The following table, giving the minimal, average and maximal doses employed in the authors' experience, may prove of service:

THERAPEUTIC DOSE TABLE

Bacteria	Minimal effective dose	Average dose	Maximum dose
Gonococcus.....	2 to 5 million	50 to 100 million	400 million
Colon.....	5 to 10 million	25 to 50 million	400 million
M. Catarrhalis.....	10 to 25 million	50 to 100 million	300 million
Influenza.....	5 to 10 million	25 to 50 million	200 million
Typhoid.....	5 to 10 million	25 to 50 million	500 million
Pneumococcus.....	10 to 20 million	25 to 50 million	300 million
Streptococcus.....	2 to 5 million	25 to 50 million	200 million
Staphylococcus.....	50 to 100 million	100 to 400 million	1000 million
B. Punctatum.....	10 to 25 million	100 to 200 million	400 million
B. Proteus Vulgaris....	5 to 10 million	50 to 100 million	400 million
B. Friedländer.....	10 to 20 million	50 to 75 million	300 million
B. Acnes.....	5 to 10 million	25 to 100 million	200 million
Pertussis.....	5 to 10 million	25 to 100 million	200 million
Diphtheria.....	5 to 10 million	25 to 50 million	100 million
Pseudodiphtheria.....	10 to 25 million	50 to 100 million	300 million
B. Pyocyaneus.....	50 to 100 million	400 million
B. Fluorescens.....	50 to 100 million	400 million
B. Pseudotuberculosis
Rodentium.....	25 million	50 to 100 million	400 million
B. Acidi Lactici.....	50 to 100 million	400 million
B. Lactis Aërogenes....	50 to 100 million	400 million
B. Mallei.....	2 to 5 million
B. Fusiformis.....	10 to 25 million	50 to 100 million	400 million
B. Koch-Weeks.....	50 to 100 million	400 million
B. Morax-Axenfeld.....	50 to 100 million	250 million
Tuberculin.....	0.00001 to 0.0001 milligram	0.0001 to 0.001 milligram	100 milligrams
Actinomycotin.....	0.001 milligram	0.01 to 0.1 milli- gram	4 milligrams

The physical and clinical state of the patient, apart from the variability in the virulence of bacteria, oftentimes presents a difficult problem as to the correct dosage of bacterin. In this connection, the age, sex, and stature of the patient, the gravity of his illness, the acuity or chronicity of the affection, a non-febrile or febrile condition and his debilitated or toxic state demand the keenest discrimination. The golden rule is *the sicker the patient the smaller the dose*, but both the clinical symptomatology and the opsonic index may suffice to determine that he is too sick for any dose, however infinitesimal.

Subsequent inoculations both as to size of doses and intervals may be governed in most diseases by close and accurate observation of the clinical symptoms or by the revelations of opsonic indices or by the utilization of both of these controls, an advisable procedure for the best results in no small number of cases. Generally speaking, the clinical symptomatology is of primary importance and the opsonic index takes second place; at times, however, these become reversed. It is an easy matter to control properly the process of immunization by study of the clinical symptoms, and success will obtain only by an experienced and careful observation of such trivial phenomena as malaise, indisposition to work or play, bodily aches, grippe-like attacks, headache, anorexia, slight fever,

nausea, variations in weight, leucocytic and hæmoglobin determinations, and particularly local manifestations in the diseased region as well as at the sites of inoculations. Allusion has previously been made to the fact that there are many diseases in which guidance furnished by determinations of the opsonic index is indispensable for the proper execution of bacterin therapy, and without which bacterial inoculations, perhaps, had best not be practiced. These include especially deep-seated affections, as bronchitis, pneumonia, certain intestinal infections and diseases of the genito-urinary tract typified by pyelitis, cystitis, seminal vesiculitis, prostatitis, etc. The safest rule to be observed respecting bacterin therapy, controlled only by the clinical symptomatology, is to begin with a very small or assuredly harmless dose; if there be absolutely no reaction, local, focal or general, in two or three days a second inoculation, twice the size of the first, may be given, and so on with intervals of three to seven days until reactionary phenomena are observed. The slightest reaction is evidence that the dose has been sufficient if not too large and indicates that no further inoculations are to be made until all reactionary signs have completely subsided for several days. Frequently, the next inoculation must be deferred for two or more weeks and when administered should not exceed in size its predecessor, commonly

being cut down to only one-half of the previous dose. Strict observance of this dictum is imperative, but unfortunately not in accordance with the instructions furnished the general practitioner by all pharmaceutical firms marketing stock bacterins, thus accounting for no small number of failures in bacterin therapy. No hard and fast rule can be dogmatically laid down, either as to interval or size of the inoculations. Each case is a study unto itself and must be treated accordingly.

Contra-indications, Limitations and Causes of Failure of Bacterin Therapy.—By virtue of the theory of biological therapeusis, little should be expected of bacterial inoculations in the acute stages of infectious diseases, indeed they may exert an evil influence. Bacterin therapy, however, is particularly contra-indicated when the individual is overwhelmed by a diffuse infection or when owing to prolonged illness he is prostrated or his tissues are greatly wasted and no longer susceptible to artificial stimulation for the production of antibodies. Such states are bacteraemia, septicæmia, pyæmia and sapræmia. Among other contra-indications should be mentioned ignorance and inexperience on the part of the would-be immunologist, and complicating surgical conditions demanding primary and immediate operative intervention.

Bacterin therapy has definite and, in the minds of immunologists, well-recognized limitations. It is not assumed to be a specific, capable of cure in all cases to the exclusion of other important time-honored therapeutic measures, physical and chemical. It purports to be merely the logical and scientific means to assist Nature in her struggle against infection, and, with due appreciation and application in this light, it will seldom fail to render due service. It offers no promise to resuscitate a medical derelict.

Allusion must be made to certain causes of failure by those practicing bacterial inoculations. Sum-
marily, they may be stated to be: (1) utilization of the improper bacterium, whether autogenous or hetero-
geneous; (2) routine employment of stock instead of
autogenous bacterins; (3) ignorance in administra-
tion, either of size of doses or intervals of inoculations, and (4) disregard of commonly associated conditions.

The mistake of utilizing the wrong germ could be obviated by the routine employment of autogenous bacterins whenever possible. This implies technical laboratory and bacteriological knowledge by each physician contemplating bacterin therapy or that he consult with or refer his patient to an immunologist. Thus there would arise no shifting of responsibility between the laboratory worker making and standardizing the bacterial suspension and the general prac-

titioner desiring to give the inoculations. Otherwise, in the event of an unhappy result, the former will accuse the latter of incompetence and the latter the former of faulty technic. This seems like stringent doctrine, but we believe in the long run will redound not only to the credit of physicians and bacterin therapy, but also and especially to the better health of patients. Even in the experience of expert bacteriologists it becomes at times no easy task to isolate from a mixed infection the fundamental etiological bacterium. Obviously, a bacterin prepared from a superimposed or secondary infection, even though that bacterium be preponderant, would produce little or no effect, curatively, upon the primary or underlying morbid process. Stock bacterins, if employed—a procedure at times advisable—should be polyvalent, that is, constituted by as many strains as may be obtainable of the particular species of bacterium in question. Caution should always be exercised to determine by bacteriological methods the exact identity of the infecting bacterium in a given case before proceeding with the “supposedly correct” stock bacterin. On more than one occasion we have found patients falsely immunized with a stock preparation devoid of the bacterium actually causing the disease. Such failures discredit physicians vastly more than the mode of therapy. The advantage of the autogenous prepara-

tion is, naturally, that the patient will be immunized with the exact strain of the germ by which he has been infected, leading to a more definite and decisive result. The commercial so-called "mixed bacterins or vaccines" for therapeutic purposes cannot be too highly condemned, simply on scientific grounds; prophylactically their employment would appear rational. It is not true that the injection of a few million extra brands of germs, in addition to that responsible for the main affection or possibly concomitant infections, is without deleterious effect. Each variety of bacteria thus injected stimulates the tissue cells to the production of its specific antibody. This, certainly, is an unnecessary work or drain on the part of the immunological mechanism, so far as the irrelevant bacteria are concerned, and has no effect therapeutically upon the pathological process. Again, the bacterin, especially if heterogeneous, may be unfit for use, that is, too old or in a state of precipitation or autolysis, or chemically contaminated from its container, be it bottle, vial, ampoule or syringe.

Finally an item of much importance in the preparation of a bacterin, whether autogenous or stock, is that it should not be overheated, thereby destroying its immunizing properties. The correct degree of temperature is merely the thermic death point for the respective bacterium.

Many failures in therapeutic inoculation result from improper administration of the bacterin. The doses given are either too large or too small, more frequently the former, and the intervals carelessly spaced. This is partially accounted for by the fact that some physicians are gifted with superior judgment and possess keen powers of observation, while others are below par, yet overworked and indifferent, or overenthusiastic or prejudiced. Another important cause of failure in biological therapeutics is disregard of associated conditions. These are both general and local. The general comprise diabetes, intestinal stasis, constipation and toxicosis. In these states phagocytosis is markedly handicapped or impossible. The local causes responsible for failure include marked suppurative foci, caries, sequestra and other conditions demanding primary surgical intervention. Again, owing to the anatomy of the part, as in chronic otitis media, also on account of the pathology as observed in sinuses, fistula and old abscess cavities, where, due to coagulated fibrin and old inflammatory exudate and cicatrization, the diseased area becomes walled off, it is impossible for the systemic blood-serum, even though highly opsonized, to bathe the affected part, hence exert a healing influence. Here the rational employment of hyperæmia, by massage, rubefacients, heat and Bier's methods, flushing sinuses,

abscesses, etc., with Wright's solution (one and a half per cent. sodium citrate in five per cent. saline), incision, citric acid to decrease the viscosity of the blood, nuclein and eliminants and tonics directed to the intestinal tract, kidneys, skin and liver, become indispensable adjuvants, converting an otherwise sure failure into natural victory.

Application and Results of Bacterial Inoculations in Special Diseases.—During the past decade, owing to the opsonic tidal wave and the resultant interest in bacterial inoculation, injections of killed bacteria have been made, both in a protective and curative capacity, in almost all infections or infectious diseases to which man falls heir. There is not a system in the human organism to which bacterin therapy has not been found applicable. Just as was the case with tuberculin therapy in the early nineties, the therapeutic pendulum swung too far, but to-day the cord is being drawn tighter and tighter around those specific diseases amenable to this form of therapy and year by year the indications and contra-indications and *modus operandi* become better known and the doctrine of bacterial inoculation more intelligently engrafted.

DISEASES OF THE SKIN AND SOFT PARTS

This group, comprising acne, boils, carbuncles, abscesses, ulcers, cellulitis, dermatitis, impetigo, syphilis, sinus, fistula, tuberculosis, glanders, actinomycosis,

smallpox and bubonic plague, by all odds furnishes the most fertile field for bacterial inoculations, therapeutically and prophylactically.

Acne.—Extraordinary results have followed bacterin therapy in this disease, especially in the pustular variety, when usually the *M. albus*, but occasionally the *M. aureus* and rarely the *M. citreus*, have been isolated (Fig. 89). Incipient or non-pustular acne,

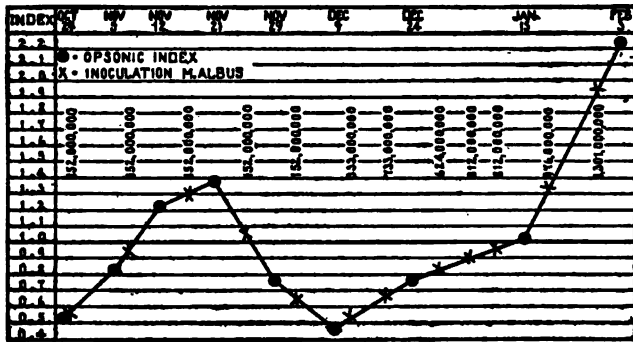


FIG. 89.—Harris E. T. Case of long-standing and obstinate pustular acne vulgaris. Yielded to autogenous bacterin therapy only after a prolonged course of treatment.

according to the researches of Unna and Sabouraud caused by the *Bacillus acnes*, is in many cases favorably influenced by inoculations with this organism, although the results are not nearly so brilliant as with the staphylococci in the pustular form of the disease. Sabouraud contends that the acne bacillus is also the cause of *seborrhæa* and *alopecia areata*, and Fleming has reported signal successes in the treatment of these conditions with *B. acnes* bacterin. In the treatment of

acne in any form, the accessory or supplemental measures are of prime consideration, and include correction of underlying systemic conditions, notably alimentary and genital disorders, lymphagogues as sodium citrate or citric acid internally and hot fomentations, the removal of comedones and pustules locally, followed by sulphur, salicylic acid or betanaphthol ointment, etc.

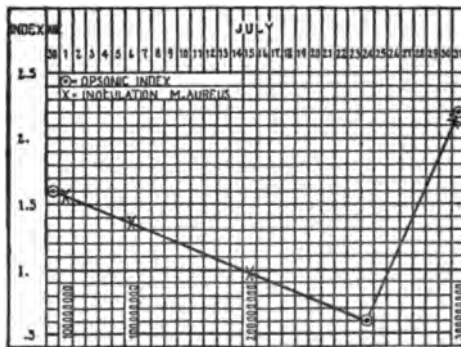


FIG. 40.—Annie H. Furunculosis of nostril. It will be observed here that the inoculations were governed entirely by the clinical course of the case; the opsonic indices were taken incidentally, merely, to note when the patient reached the "high tide of immunity," so that further inoculations might be suspended.

Furunculosis and Carbunculosis.—None the less remarkable are the effects of bacterial inoculations in these common affections. The infecting bacterium is almost invariably the *M. aureus*. In the case of the acute primary boil or carbuncle, bacterin therapy need not and should not be used except by an expert possessing a knowledge of the opsonic index, since more harm than good may result (see page 226), whereas

in the chronic recurrent form of the diseases it is the most valuable therapeutic agent at our disposal. Here again opsonic indices utilized to guard against over-inoculation, and to govern remote inoculations, will suffice to prevent recurrences (Figs. 40 and 41).

Abscesses.—These may be primary or complicate other diseases, as typhoid fever. The bacteria isolated include *M. aureus*, *albus* and *citreus*, *Streptococcus pyogenes*, *Pneumococcus lanceolatus*, *B. coli*, *B. ty-*

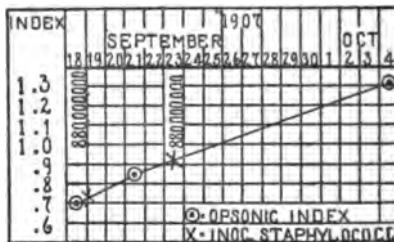


FIG. 41.—Fred G. Carbuncle of neck. Observe in this case the utilisation of the opsonic index to determine the maintenance of immunity in the "positive phase," after the suspension of inoculations.

phosus, *B. pyocyaneus*, *B. tuberculosis*, *B. anthracis*, *B. mallei* and *Streptothrix actinomyces*. An autogenous bacterin is always preferable to a stock preparation and the therapy when so conducted usually results brilliantly (Figs. 42 and 43). Little should be expected from bacterial inoculations with secondary invaders, as *B. pyocyaneus*, *B. lactis aërogenes*, etc., or with *B. anthracis*, *B. mallei* and *Streptothrix actinomyces*.

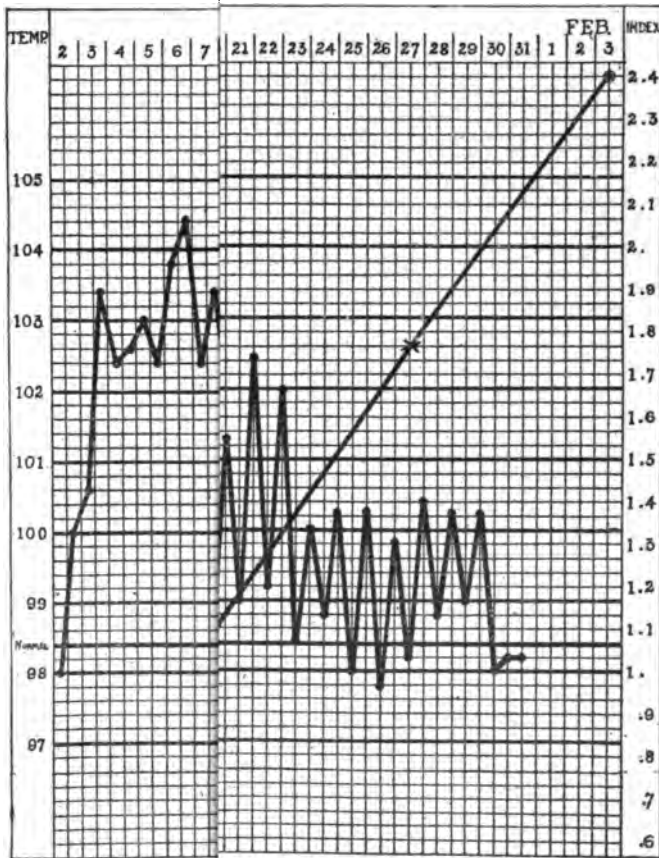


FIG. 43.—Linda C. represents opsonic indices. A, skin grafts to arm and leg; B, development of abscesses demonstrated *M. aureus*. The small crosses on the diagonal line indicate condition and health of the patient. Note also that no new abscesses formed.

Ulcers.—A variety of bacteria have been identified, the majority of which in chronic and intractable

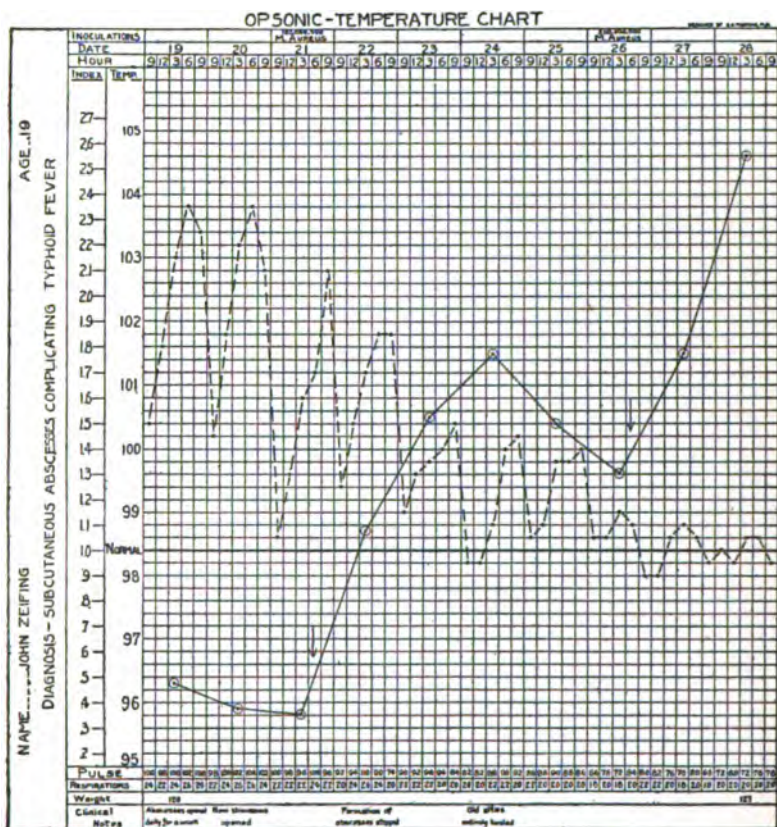


FIG. 42.—In this case of typhoid fever a few days after the return of the temperature to normal during convalescence, dozens of subcutaneous abscesses formed persistently for a week or more, requiring daily incisions and drainage. Cultures demonstrated *M. aureus* in pure state. Observe the immediate effect of a single inoculation of 100,000,000 staphylococci. Coincidentally with the fall in temperature and general improvement, the opsonic index showed a marked rise.

cases arise as secondary infections, against which bacterins may possess little value unless the primary in-

fection, as tuberculosis, syphilis, etc., can be influenced. In addition to all of the common pyogenic bacteria, the following should receive consideration: *B. fusiformis*, *B. punctatum* and certain corynebac-

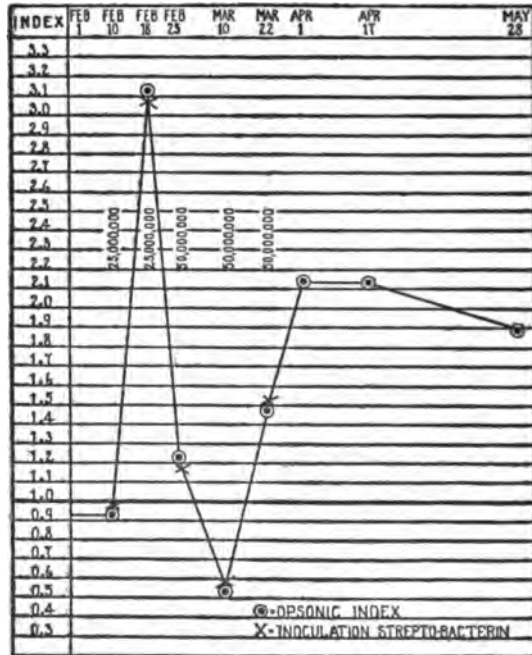


FIG. 44.—Mrs. J. B. C. Recurrent erysipelas. Observe in this case that the "high tide of immunity," indicated by the opsonic indices, has been maintained for two and a half months, no inoculation having been given since March 22. It will be noted that this is entirely in accord with the clinical symptoms of the case.

teria. In the treatment of rapidly spreading phagedenic ulcerations, excellent results have been obtained. Naturally, autogenous preparations should be utilized whenever possible, and cultures repeated frequently as the bacterial flora are subject to change.

Cellulitis and Dermatitis.—Under these headings are included *erysipelas*, *Ludwig's angina* and *pemphigus*, commonly associated with *lymphangitis* and *lymphadenitis*. The preponderant invading organism is the streptococcus, although the staphylococcus is also found. Streptococcal infections of this class, in

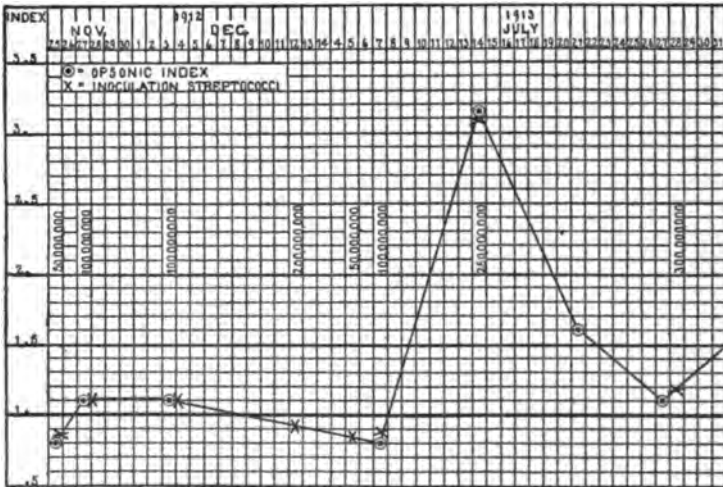


FIG. 45.—Mrs. C. Tonsillitis, peritonsillitis and toxic arthritis. Even after deep cervical incisions through an extensive cellulitis to the tonsillar region for drainage, the patient did not progress satisfactorily until the administration of an autogenous bacterin, which exerted a specific and immediate beneficial effect both on the cervical infection and also on the arthritic manifestations.

the acute stage, run a short course immeasurably influenced by bacterins; in the subacute, chronic or recurrent stages, bacterial inoculations have proved of immense benefit, at times performing the incredible (Figs. 44 and 45).

Impetigo and Sycosis.—The invading microbe

may be either the staphylococcus or streptococcus, consequently culture and the appropriate autogenous bacterin may be employed with benefit. In impetigo the result is uniformly successful; in sycosis treatment, oftentimes prolonged, should result in fifty per cent. cures, the remainder showing improvement.

Sinus and Fistula.—Various bacteria, as the *Staphylococcus*, *Streptococcus*, *B. coli*, *B. proteus vul-*

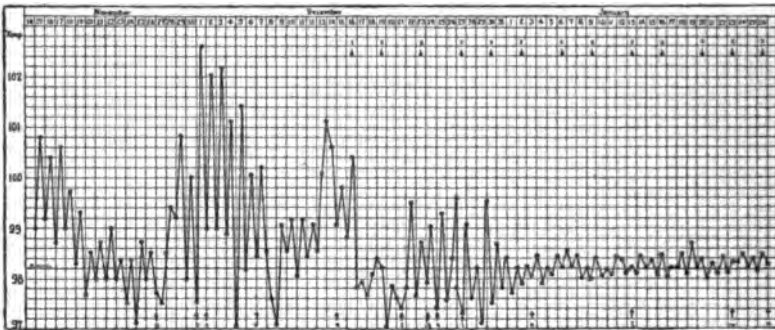


FIG. 46.—Temperature. Patient, W. F. Subdiaphragmatic abscess drained per laparotomy: a, patient very comfortable, drainage satisfactory; b, patient complains of pain in hepatic region; c, leucocytes 20,000; d, excessive suppuration; e, suppuration variable but very profuse; f, patient's appearance improved, feels much stronger; g, patient discharged from ward; h, treated in out-patient department; i, suppuration markedly decreased; k, discharge from wound very slight; l, marked gain in strength and health; m, no discharge; n, sinus healed; o, pus demonstrates *M. aureus* and *albus*; p, 2 inoculations *M. aureus* bacterin $\frac{1}{4}$ c.c. This case illustrates how admirably active immunisation can be conducted by resort to the clinical symptomatology as a guide, to the exclusion of the opsonic index.

garis, *B. tuberculosis*, *B. pyocyaneus*, *B. fluorescens*, *B. lactis aërogenes*, etc., have been found. The results in fistulæ have been unsatisfactory; in sinuses, if no surgical indications exist and lavage of the tract with Wright's solution to promote osmosis and hyperæmia is used to facilitate the effectiveness of therapy,

bacterins and tuberculins in many cases have possessed considerable accessory value (Figs. 46 and 47). Our experience with *pyocyaneus*, *fluorescens*, and *lactis aërogenes* bacterins in any condition has never been glorifying. Tuberculosis commonly involves the skin and subcutaneous tissues, especially the lymph-nodes, and is notably associated with sinuses and fistulæ.

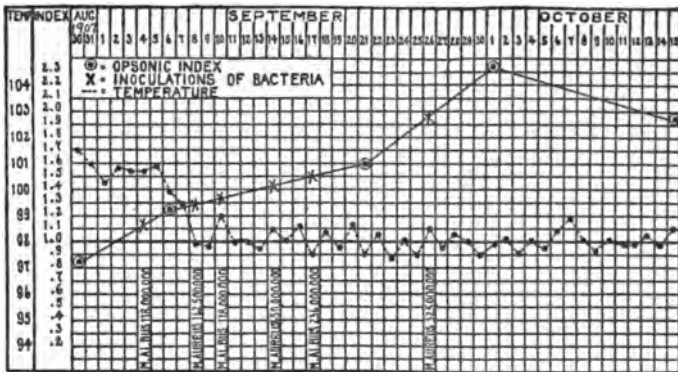


FIG. 47.—Mrs. B. Pelvic abscess with recto-urethro-vaginal fistulæ. Note the fall of temperature to normal as the curve of opsonic antibodies rises.

The reader is referred to Chapter XVI, where the inoculation treatment of this disease is detailed.

Actinomycosis.—Actinomycoses may involve the lungs, liver, kidneys, bladder, brain, abdominal viscera, bones, genitalia or subcutaneous tissues. Actinomycotic inoculations in the pulmonary, hepatic, renal, vesical and cerebral forms of the disease are of little or no value. Actinomycotin, standardized so that one cubic centimetre contained one milligramme of the

sterilized powdered organisms, we have found beneficial in superficial localized lesions, using an initial dose of 0.001 milligramme.

Glanders.—Bacterins of *B. mallei* or “mallein” have been attended with indifferent success and in a generalized infection of glanders offer no promise of cure whatsoever.

Variola.—Smallpox, the greatest and most loathsome scourge that ever decimated the human race, is to-day a disease of comparative rarity. With an incidence of ninety-five per cent. among the exposed and a mortality of twenty per cent., in the eighteenth century it was estimated that 2,000,000 people in Europe annually contracted variola, of whom 400,000 died. Jenner's vaccination against smallpox in 1798—the first and classical example of the prophylactic employment of bacterial inoculation—has resulted almost in the extermination of this disease. Physicians of eminence in every civilized country of the globe fully realize the inestimable value of this achievement for mankind and appreciate the fact that vaccinia properly employed protects against variola. In Germany, where the most advanced and adequate legislation has been enacted for forty years, compulsory vaccination and revaccination have rendered security against smallpox almost absolute. Originally crusts from variola ulcers, then crusts from the vaccinia

sores of otherwise healthy individuals, were utilized. Later experiments on calves showed that smallpox virus can be converted into vaccine virus by passage through several (four) generations of the bovine species. Thus the virus is rid of any possible contamination from human sources and can be and is prepared in any desired quantities. The best vaccine virus is glycerinized and a number of reliable pharmaceutical firms market the lymph in sealed capillary glass tubes, protected vaccine points and vials. The virus must be preserved in a refrigerator.

Technic of Vaccination.—The skin of the arm preferably or the leg is cleansed with alcohol or ether. The superficial layer of the epidermis is denuded by a sterile von Pirquet scarifier or sharp dental chisel by a rotary motion, or in the absence of these by a scalpel or needle, producing an area about two millimetres in diameter, caution being observed to draw only serum, not blood. The scarification may be done through a drop of virus primarily placed on the skin or the virus may be deposited when the denudation is completed and rubbed in with a sterile orange stick or toothpick. If possible the drop of lymph should be allowed to dry in the air or after fifteen minutes a gauze dressing or vaccine shield may be applied to the inoculated site for protection. The cow-pox wound should be inspected daily for at least five days.

Characteristics of Vaccinia Sites.—Due to trauma alone, a few minutes after vaccination the skin immediately surrounding the wound assumes a reddish line; this disappears in a few hours. If at the end of twenty-four hours the vaccinated spot exhibits an areola, with or without papule, one-half centimetre or more in diameter, which inflammatory zone decreases in seventy-two hours, it is to be regarded as an *allergic reaction of immunity* due to specific antibodies in the individual and excuses him from further immediate vaccination. If the areola observed at the end of twenty-four hours develops into a small vesicle maturing on the fifth or sixth day, then rapidly subsiding, it is to be regarded as a case of *vaccinoid*, that is, one where, although antibodies are not present, the power to form them exists from previous vaccination. If reactionary changes, characterized by a reddened, inflammatory, indurated areola or aula surrounding a papule, supervened only from the second to the fifth day, followed by flattening of the summit of the papule, it is to be regarded as a case of typical *vaccinia*. By the tenth day the reaction will have reached its maximum, the inflammatory areola measuring sometimes more than five centimetres and the papule as much as one centimetre. The papilla at the onset assumes a pale pinkish-gray color, changing to a gray-

ish-yellow, followed by rapid drying and crust formation. Involution begins usually about the tenth day and by the end of the second week only a thick, brown, tightly adherent crust surrounded by pigmented skin marks the original site of the papule.

From the standpoint of therapy it is of interest to note that recently autogenous staphylococco-bacterins prepared from the pustules of smallpox have been successfully used in diminishing the degree of cutaneous pitting.

Bubonic Plague.—Treatment with bacterial suspensions of *B. pestis* has been extensively practiced by Haffkine. Prophylactic inoculation is of much greater value than therapeutic administration, and has reduced the mortality from 66.6 to 16.6 per cent. in the experience of Strong, who employed living attenuated bacilli. The primary immunizing dose should be five billion bacteria, followed in ten days by ten billions. Immunity is alleged to endure from three to eighteen months. Therapeutic inoculation greatly reduces the severity of attacks, and recovery always occurs if the victim be a European. Curatively, however, Yersin's or Lustig's antitoxic serum supersedes bacterin as a therapeutic measure. Bacterins are probably valueless in the septicæmic and pneumonic forms of the disease.

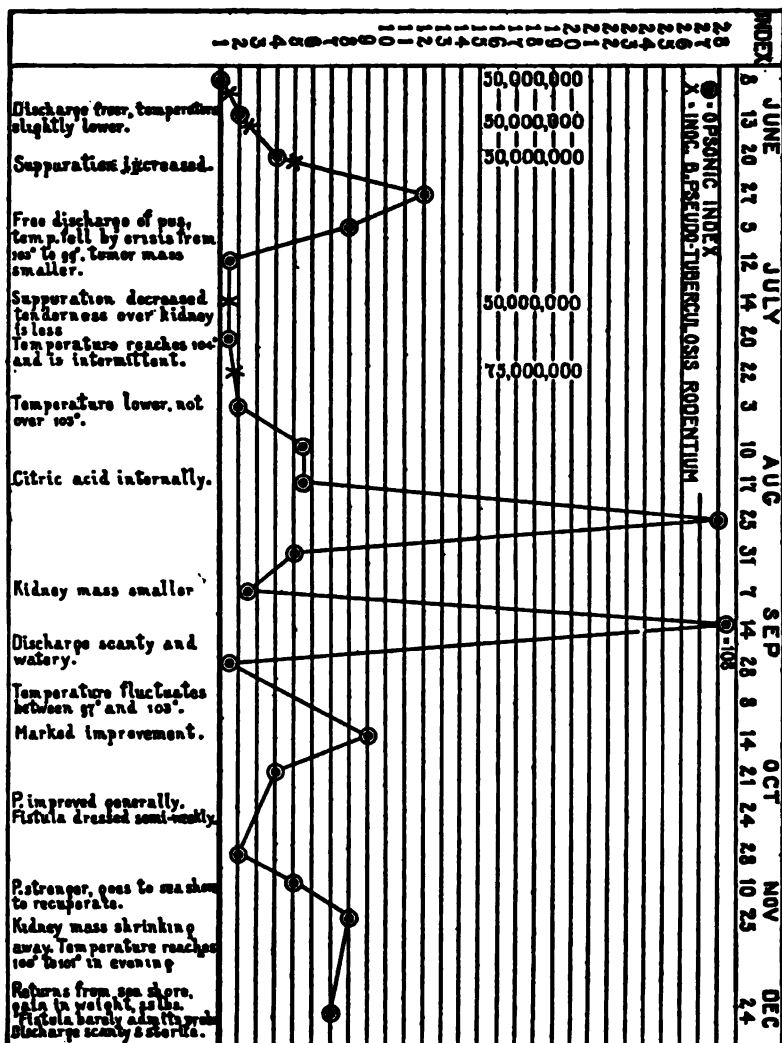


FIG. 48.—Dorothy W. Pyonephrosis. Nephrectomy in this case was impossible owing to the size and adherent state of the kidney. Nephrectomy temporarily relieved for a few months; then the patient became critically ill and was rescued apparently by autogenous bacterin therapy. Note the unprecedented high flights of the opsonic index, especially that of September 14.

DISEASES OF THE GENITO-URINARY SYSTEM

Cystitis, Pyelitis, Pyelonephritis and Pyonephrosis.—Bacterins from the following microorganisms have been of service in certain diseases of the kidney and bladder: *B. coli*, *B. tuberculosis*, *B. pseudotuberculosis rodentium*, *B. of Friedländer*, *B. typhosus*,

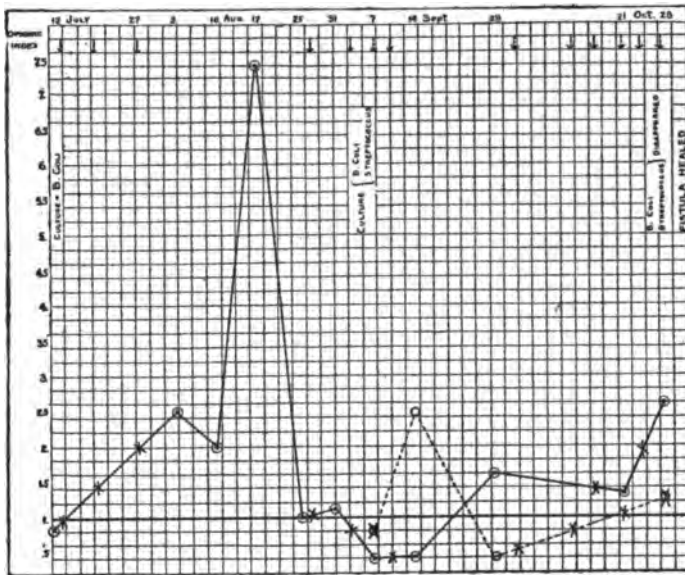


FIG. 49.—Albert P. Reno-lumbar fistula following nephrolithotomy complicated by pyonephrosis. Note occurrence of a mixed infection in August, demonstrating the advisability of autogenous bacterins and the necessity of re-culture at intervals. —→ = inoculation with colon bacilli; ---→ = inoculations with streptococci.

Streptococcus pyogenes, *Staphylococcus* and *M. lanceolatus*. Great discrimination must be exercised as to whether the particular condition is amenable to bacterin therapy or should be operated upon. Bacterins were never intended to supplant surgery, but at times

are wonderful accessory agents in promoting cure, and not infrequently, with proper administration, relieve surgery of some of its indications. In colon bacillus infections of the bladder and kidney in children, also in kidney suppurations where, owing to the size and adherent character of the diseased organ,

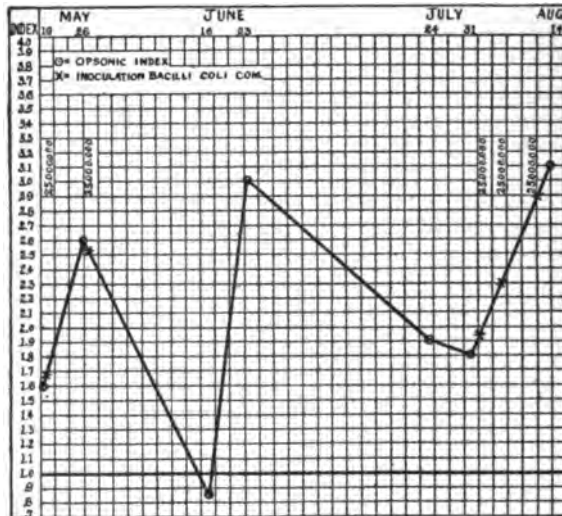


FIG. 50.—Margaret B. Pyelitis and cystitis.

nephrectomy is temporarily impossible, autogenous bacterins have proved signally meritorious (Figs. 48 and 49). In such deep-seated infections and in cystitis and pyelitis estimations of the opsonic index to control the inoculations have been invaluable (Figs. 50 and 51).

Urethritis.—Compared with the gonococcus of Neisser all other invading microorganisms sink into

insignificance, although occasionally a non-specific infection arises, due to the *M. catarrhalis* or other pyogenic bacteria. Gonococcus bacterin has been utilized both prophylactically and therapeutically against gonorrhœa. As a protective measure, Douglas, Wright's associate, recommends an initial dose of 100,000,000. If this is followed by no reaction a second inoculation of 200,000,000 is administered in a week or ten days. The adoption of this procedure is

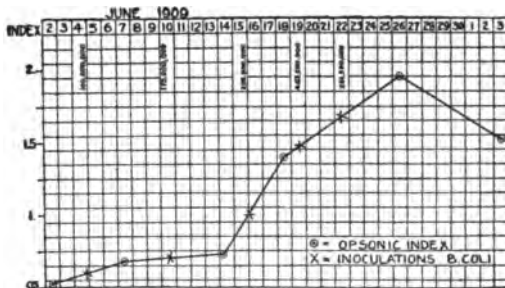


FIG. 51.—Cystitis and toxic neuritis.

advisable, although unattended with any absolute assurance, in certain marital relations when the disease is innocently acquired, for the protection of girls in an institution in the event of an outbreak of gonorrhœal vulvovaginitis, and perhaps to safeguard innocent newlyweds, under peculiar circumstances, when one of the contracting parties has recently convalesced from an attack of urethritis.

Again, it must be borne in mind that the discharge

of an acute gonorrhœa, sooner or later, invariably contains bacteria other than the gonococcus, namely, the *Streptococcus pyogenes*, the *Staphylococcus*, the *Pneumococcus*, the *Colon bacillus*, the *Micrococcus catarrhalis*, etc. Thus the prophylactic effect of early immunization in a case of gonorrhœa against these complicating germs, in the prevention or amelioration of such conditions as gleet, prostatitis, seminal vesiculitis, etc., should not be underestimated. The authors feel that in many cases in their experience the cautious administration of mixed gonococcic bacterin has sufficed to shorten convalescence and to prevent or reduce the severity of certain complications, notably inflammation of the prostate and seminal vesicles and stricture formation. It is a difficult or impossible task to gauge the effect of treatment upon any disease prone to run a definite course, consequently the value of bacterins in urethritis, in numberless instances, has been immeasurable, and although certain investigators have reported favorably on biological therapeutics in acute gonorrhœa, consensus of opinion discourages the procedure.

In this connection reference must be made to Bruck's "Arthigon," a suspension of dead gonococci, standardized to contain twenty millions per cubic centimetre. Trustworthy evidence has accumulated to the effect that the intravenous injection of this

preparation in quantities of 0.05 to 0.5 c.c. produces a reaction which is invariably constant in gonorrhœal subjects. A rise of temperature above 1.5° C. after an injection of 1 c.c. may be regarded as pathognomonic. Therapeutically, with increasing dosage, the results have been no better than with the subcutaneous administration of bacterins or sero-bacterins, and it is extremely doubtful if intravenous injection will supersede the older method, properly interpreted, in a diagnostic capacity.

Prostatitis and Seminal Vesiculitis.—The writers believe that these complications, in addition to the gonococcus, are commonly precipitated and perpetuated by a mixed infection due to the *Streptococcus*, *Pneumococcus*, *Colon bacillus*, *M. catarrhalis*, etc. They have obtained in many instances gratifying results by alternating a decivalent gonococcus stock and autogenous bacterins, the latter prepared from urine cultures after massage of prostate and seminal vesicles, when, in addition to the above, other organisms, as the *Micrococcus albus*, *aureus*, *citreus*, *candicans*, *candidus* and *orbicularis*, the *Bacillus typhosus*, *pyocyaneus* and *acidi lactici* and the *Corynebacterium pseudodiphtheriticum*, have been isolated. The therapy has been conducted only in the subacute and chronic cases, preferably in the presence of suppurative inflammations (Fig. 52).

Epididymitis.—*Gonococcus* bacterin is probably of value in acute epididymitis (Fig. 53), although the disease is prone to run a definite short course under proper treatment without immunotherapy. It would appear, however, that the employment of bacterin di-

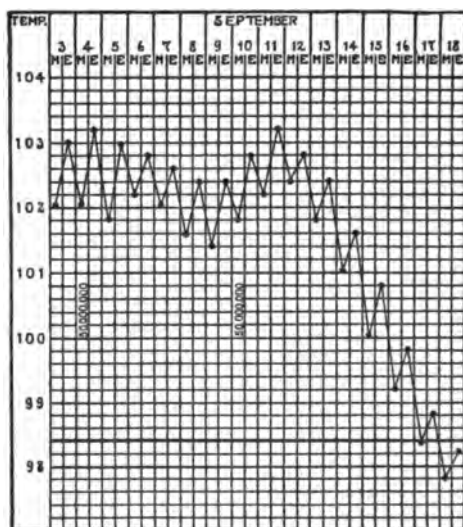


FIG. 53.—Typhoid fever complicated by epididymitis. Observe transient slight fall in temperature a few days after first inoculation of typho-bacterin, and the fall by lysis after the second dose of bacterin.

minishes the likelihood of a resultant inflammatory nodule of the epididymis, at the same time reducing the liability to sterility. In the chronic form due to tuberculosis, tuberculin is of unquestionable value, both diagnostically and therapeutically, although in no small numbers of cases epididymectomy must be

performed, and this applies particularly to dispensary patients.

Vulvovaginitis.—In children inoculations of a polyvalent gonococcus bacterin, in the acute stage, have resulted brilliantly, causing the early disappearance of gonococci and shortening the usual course of treatment by months. Five million gonococci constitute the average dose. From three to a dozen inoculations are required. Benefit is also observed from autogenous bacterins in the chronic form of the disease due to other pyogenic bacteria, as the *Streptococcus*, *Staphylococcus*, *Pneumococcus* and *Colon bacillus*. Vaginitis in adults, in the experience of the authors, has been absolutely uninfluenced by bacterial inoculations.

Cervicitis, Endometritis, Metritis and Salpingitis.—The following bacteria have been isolated: *M. gonorrhææ*, *B. coli*, *Pneumococcus*, *Streptococcus*, *Staphylococcus* and *Tubercle bacillus*. This category of diseases needs further investigation respecting the value of bacterins, since the studies to date have been inconclusive. In general the results have been very indifferent, scarcely warranting the procedure. This is certainly true in the case of cervicitis. In the treatment of salpingitis a number of remarkable results have been reported. An essential to success lies in the

selection or preparation of the appropriate bacterin, dependent upon the determination of the invading microbe.

Puerperal sepsis is entitled to special and serious consideration. In our opinion the applicability of bacterins is directly dependent upon the presence or absence of a general bacteræmia, determinable by blood culture. If the former exists, active immunization is definitely contra-indicated; if the septic process is localized to the pelvis, bacterial inoculation, cautiously employed, may prove serviceable. We have been gratified on several occasions by the excellent results of bacterins supplementing or alternating passive immunization (serum therapy). In any event, this is a condition wherein opsonic indices will render important service in the control of therapy.

DISEASES OF BONES AND JOINTS

Osteitis, Periostitis and Osteomyelitis.—Infections from the following bacteria come into consideration: *Staphylococcus*, *Streptococcus*, *Pneumococcus*, *Gonococcus*, *Typhoid bacillus*, *Colon bacillus* and *Tubercle bacillus*. In the acute stage of an osteoperiostitis, if bacteræmia is not present, bacterin therapy is very beneficial, especially in pneumococcic, gonococcic, typhoid and colon infections; in early spur formation

from the os calcis (osteophytes, exostoses) the administration of gonococcus bacterin has rendered operative intervention unnecessary, or at least has eliminated toxins and insured a more satisfactory post-operative result. In chronic cases it is even more

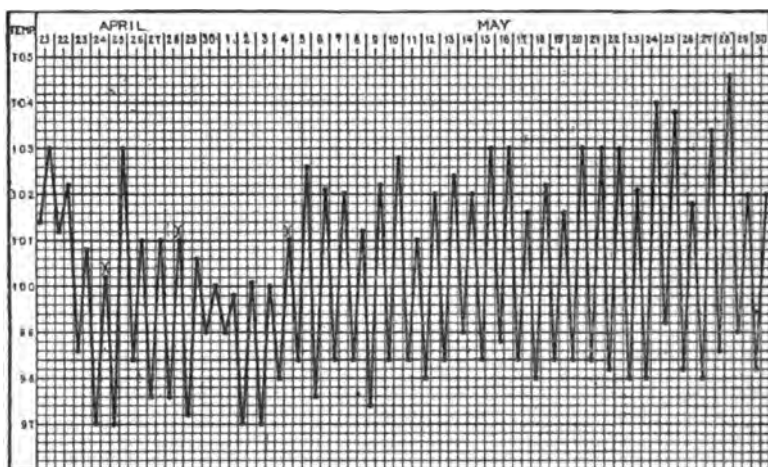


FIG. 54.—Sam. S. Acute osteomyelitis of tibia followed by septicæmia. The arrow indicated when a blood culture demonstrated *M. aureus*; the crosses represent the times of inoculations with *M. aureus* bacterin. Observe that the patient, as indicated by the increased fluctuations of temperature, progressively grew more septic, and that bacterin therapy was not only useless, but possibly harmful. The substitution of bacterin at the juncture by antistaphylococcic serum apparently exerted a definite beneficial influence for recovery.

meritorious, if caries and sequestra are excluded and hyperæmia used to augment the treatment. In our experience bacterins and tuberculins have done more harm than good in acute osteomyelitis (Fig. 54).

Synovitis and Arthritis.—Associated with these diseases the *Streptococcus rheumaticus*, *Gonococcus*,

Pneumococcus, *Streptococcus pyogenes* and *Staphylococcus* should be mentioned. Bacterins compiled from the first-named organism, whether isolated from the joint, gums, tonsils or fæces, have in a number of instances rendered therapeutic service in the acute, subacute and chronic forms of disease. We have never observed any remarkable benefit by the employment of the common pyogenic streptococci and staphylococci, nor is much to be expected in chronic cases where hypertrophic osseous changes have occurred. On the other hand, this is a field wherein gonococcus bacterin enjoys much distinction. Indeed, biological inoculations furnish the treatment *par excellence* for gonorrhœal synovitis and arthritis. Many patients, with limitation of motion in subacute and chronic gonorrhœal joints, are best treated by preliminary inoculations of Neisser bacterin a few weeks or months prior to forcible manipulation under general anæsthesia, thereby eradicating the cause of the inflammatory exudate and preventing its re-formation after operation.

It should also be observed in this connection that gonococcus bacterin is of exceptional value in the differential diagnosis of many obscure joint and other gonorrhœal afflictions, used precisely as is tuberculin in a diagnostic capacity.

DISEASES OF THE EYE, EAR, NOSE AND THROAT

Singularly perhaps, but nevertheless fortunately, infections of the eyes have, in the majority of instances, demonstrated the great value of bacterin therapy; not so much can be claimed in the treatment of diseases of the ear, nose and throat. There are certain anatomical facts explanatory, at least in the case of the ear, of these variable effects.

Conjunctivitis and Dacryocystitis.—The following bacteria have been isolated: *Pneumococcus*, *Streptococcus*, *Staphylococcus*, *Gonococcus*, *M. catarrhalis*, *Friedländer's bacillus*, *Koch-Weeks bacillus*, *Morax-Axenfeld bacillus*, *Pyocyaneus bacillus* and *Tubercle bacillus*. In many acute infections, notably that due to the bacillus of Koch-Weeks, the usual ophthalmological treatment will prove all-sufficient, while in chronic types, as that caused by the bacillus of Morax-Axenfeld, frequently little response follows ordinary treatment and the effect of bacterins appears to be specific. Between these are a number of infections due to the gonococcus, pneumococcus, streptococcus, etc., which may do well with ordinary measures, but in which the accessory employment of bacterins hastens convalescence, diminishes the inroads of the pathological process, prevents sympathetic ophthalmia and avoids the loss of vision. In

dacryocystitis bacterin therapy may obviate surgical extirpation of the lachrymal sac.

Corneal Ulcer and Hypopyon.—The *Diplococcus pneumoniae*, the *Streptococcus mucosus*, the *Streptococcus pyogenes*, the *Staphylococcus*, the *Gonococcus* and *Tubercle bacillus* have been identified. Autogenous bacterins prepared from the pneumococcus and streptococcus in cases of *ulcus serpens* of the cornea, with and without hypopyon, notably in one instance in which panophthalmitis threatened complete destruction of the eye, have produced incredible results (Fig. 55). Care must be observed not to employ too small dosage in the treatment of these affections. The initial inoculation should be at least 100,000,000 bacteria.

Iritis and Uveitis.—Here as in corneal ulcer the same organisms are found at work. If the infecting bacterium be determinable the results are invariably excellent. Especially noteworthy is bacterin therapy in gonorrhœal involvements. In these conditions, as in practically all gonococcic complications, practicability demands that a polyvalent stock preparation be employed.

Otitis Media and Mastoiditis.—An extensive array of bacteria as the *Streptococcus*, *Staphylococcus*, *Pneumococcus*, *Colon bacillus*, *B. proteus vulgaris*, *B. lactis aërogenes*, *B. pyocyaneus*, *B. fluorescens*, *B.*

diphtheriæ and *pseudodiphtheriæ*, *M. catarrhalis*, *B. influenza*, *B. typhosus* and *B. tuberculosis* have been found. Due to the fact that in the majority of cases the cultured organism is a secondary invader, also

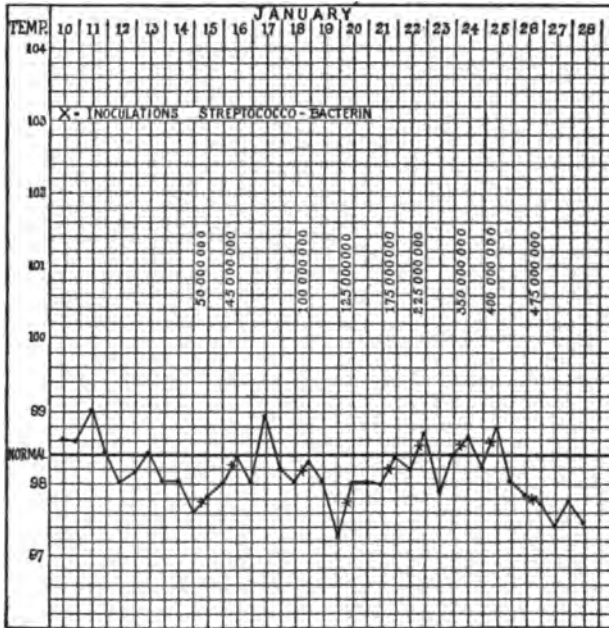


FIG. 55.—V. S. Corneal ulcer with hypopyon. Culture of pus from hypopyon demonstrated the *Streptococcus mucosus*, from which an autogenous bacterin was prepared. We believe that this patient was over-treated, that is, inoculated at too short intervals. Although he made a satisfactory recovery, it is probable that the result would have been more brilliant with less frequent inoculations.

that the infected area is encased by bony walls limiting the supply of blood, although highly opsonized, the results of bacterin therapy have been poor, especially in chronic cases. Nevertheless, in the early stage of otitis media complicating scarlet fever, the sup-

puration seems to be markedly curtailed and convalescence proportionately shortened. On the other hand, tuberculin in carefully selected cases of ear and mastoid tuberculosis has proved of great benefit.

Rhinitis and Sinusitis.—The bacterial flora of the nasal passages and associated sinuses differ little from those of the auditory apparatus. The results following the use of bacterins, however, are much better, due probably to the greater vascularity of the parts affected. Indeed, in suppurative sinusitis the employment of autogenous bacterins, aside from any surgical indication, should constitute the sheet-anchor in treatment (Fig. 56). The chief offending bacteria are *Streptococci*, *Pneumococci* and *Micrococci catarrhalis*. The dosage frequently must be increased over the average.

Laryngitis and Tracheitis.—The following bacteria are commonly demonstrable: *M. catarrhalis*, *D. pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus*, *M. paratetrigenus*, *B. diphtheriae*, *B. influenzae* and *B. tuberculosis*. Occasionally, excellent results have attended the use of bacterins in these affections, particularly with the *M. catarrhalis*, *Pneumococcus*, *Streptococcus* and *Staphylococcus*.

Diphtheria.—In recent years active immunization against diphtheria has been studied extensively by several observers. If those who are naturally immune,

[illegible]

antitoxin. In the first place, the formation of protective antibodies is slow, seldom before the second week, hence the superiority of immune serum in epidemics, both prophylactically and therapeutically. Park and Zingher point out that there is lack of a sufficient response to active immunization in at least

fifty per cent. of patients susceptible to diphtheria and that the immunity conferred lasts not more than a year or two. An important conclusion of their work is that the Schick test will demonstrate the futility of immunization in approximately two-thirds of those exposed to diphtheria.

Respecting the value of treatment of so-called "diphtheria carriers" by bacterins, opinion is divided. Some remarkable results have been reported and the procedure deserves further trial before conclusions are drawn.

Scarlet Fever.—Opinion has grown to the effect that if the streptococcus is not the cause of, it is materially concerned in the pathogenicity of the complications of scarlatina. Experience has shown that bacterins of the *Streptococcus conglomeratus*, whatever may be their effect curatively in the acute course of the disease, have unquestionably favorably influenced the complications, as angina, suppurative rhinitis, lymphadenitis, nephritis, otitis, mastoiditis and arthritis. Most remarkable are the prophylactic inoculations used by Gabritchewsky in Russia, where in thousands of cases specific immunity has been secured on the average for eighteen months. Donilow states that only 1.1 per cent. of inoculated persons contracted the disease, including those already in the incubation stage; furthermore, that the mortality

among the inoculated group was only 0.123 per cent. as compared with 11.1 per cent. among the uninoculated. For protective inoculation three doses of 250, 500 and 1000 million, from a week to ten days apart, should be administered.

Hay Fever.—An allied treatment by antigenic inoculations is that of hay fever by injections of the extracted pollen of rag-weed, on the assumption that this affection is a pollen toxicosis and not an anaphylactic expression, a thought that has gained credence recently. Noon and Freeman gauged dosage by the conjunctival reaction and injected their patients at intervals of three to ten days with units representing the amount of extract from 0.001 milligramme of pollen. Clowes was able to raise the resistance a thousandfold, although it persisted but five months, by inoculations of one cubic centimetre of 1 : 5,000,000 to 1 : 500,000 suspensions.

Recently, Lowdermilk reported astonishing results with pollen toxin, prepared as follows: One gramme of mixed pollen (250 milligrammes *Ambrosia artemisiæfolia*, 250 milligrammes *Ambrosia trifida* and 500 milligrammes of various varieties of solidago) was mixed with ten grammes of sterile sea-shore sand in a mortar, moistened with a part of a solution of 100 c.c. physiological saline containing 0.5 per cent. phenol and ground for several

hours, slowly adding the remainder of the saline-phenol solution until the sand was reduced to an impalpable powder. The whole was transferred to a sterile flask and allowed to stand with frequent shaking, at room temperature, for twenty-four hours. After decantation and centrifugation the toxin was sealed in glass ampoules, containing one cubic centimetre each. This product was standardized so that the unit represented the quantity of toxin extracted from 0.000,001 gramme of pollen. Thus each cubic centimetre contained ten thousand units. Six to eight inoculations were administered at intervals of one to ten days apart, representing from 25 to 1000 units each. The typical reactionary phenomena simulate the symptoms of an acute attack of hay fever. It is wise to reinforce the pollen toxin injections with inoculations of autogenous bacterins prepared from the complicating infection. Such treatment has been applied therapeutically as well as prophylactically. It is, however, of such recent date that no definite conclusions should be drawn. It may share the same fate as Dunbar's "pollantin" used to produce passive immunity in pollenosis.

DISEASES OF LUNGS

Bronchitis.—The following bacteria may be identified: *M. catarrhalis*, *M. paratetragenus*, *B. influenzae*, *Streptococcus*, *Staphylococcus*, *B. typhosus*, *B. coli*, *D.*

Pneumoniæ, Diphtheroid bacilli, B. tuberculosis, Friedländer's bacillus and Streptothrix actinomycosis. Pulmonary diseases offer an exceptional field for bacterin therapy, owing to the unusual vascularity of the tissues, permitting the opsonins to become effective. Consequently, brilliant results are achieved routinely both curatively and prophylactically (Fig. 56). This, of course, does not apply to tuberculosis and actinomycosis nearly to the degree that it does to other infections.

Pneumonia.—Essentially the same bacteria are to be found as in bronchitis. Keen discrimination must be exercised not to employ bacterins if there exists a doubt of bacteræmia, otherwise the employment of autogenous bacterins in adequate dosage will lessen mortality and shorten convalescence. The opsonic index here as in bronchitis should be utilized to secure the best results. In unresolved pneumonia and bronchopneumonia of childhood a special field of usefulness is offered autogenous bacterins. Prophylactic immunization applies in pneumonia the same as in bronchitis and in the event of epidemics should be routinely practiced. The protective dose is 1,000,000,000 pneumococci.

Purulent Bronchiectasis and Pulmonary Abscesses.—The bacteriology differs in no respect from that of bronchitis and pneumonia. The expectoration

must be cultured and recultured frequently for the preparation of the correct autogenous bacterins. The opsonic index will render service in the proper management of the case. Although some benefit attends the use of bacterins, the results are not brilliant, owing to the poor drainage in these chronic conditions.

Whooping-cough.—Although the bacillus of Bordet-Gengou is generally admitted to be the cause of pertussis, early in the attack, even before the second week, other bacteria as *M. catarrhalis*, *B. influenzae*, *Pneumococcus* and *Streptococcus* complicate the infection. Sufficient data have been gathered to demonstrate the value, prophylactically and therapeutically, of mixed bacterins in this disease. Indeed the attacks have not only been ameliorated but the period of convalescence shortened fifty per cent.

DISEASES OF ALIMENTARY SYSTEM

Pyorrhæa Alveolaris and Tonsillitis.—The organisms usually found include the *Pneumococcus*, *Streptococcus*, *Staphylococcus*, *M. catarrhalis*, *spirochætæ*, *vibrios* and *Tubercle bacillus*. The results of bacterin therapy have not been such as to place it as a recommendable procedure of any great moment in these diseases, unless supplemented by adequate local treatment, in the case of pyorrhæa alveolaris requiring the coöperation of a dentist. Some form of the streptococcus can be cultured in at least 75 per cent.

of cases and when remote secondary joint involvements arise and anæmias supervene, autogenous bacterial inoculations assisting effective oral treatment are of great value. It will be remembered, however, that reinfections are prone to occur, also that unless the patient is willing to submit to dental coöperation and prolonged treatment, inoculations of bacterins will prove of little or no value. In advanced disease, when the gums are spongy and necrosed, the teeth loose and the alveolar tissue in a carious state, benefit may accrue, but cure, in its strict sense, is no longer possible.*

Acute tonsillitis furnishes no particular opportunity for bacterin therapy. The importance of the tonsils as atria of infection in certain synovial arthritic and other affections must not be overlooked. Consequently in subacute and chronic disease, in peritonsillitis or in aborting attacks of quinsy autogenous bacterins may render signal service (Fig. 45). Surgery, however, is indicated in many instances, in preference to bacterins.

Enterocolitis.—Exclusive of typhoid fever, dysentery and cholera, the bacteria demonstrable in this condition, and more particularly mucous colitis, are the colon group, the *Streptococcus*, the *Pneumococcus*,

* Recent evidence points to *Entamoeba buccalis* as an etiological factor in many cases of pyorrhœa alveolaris. In these cases the local and hypodermic use of emetin hydrochloride has a markedly beneficial effect.

the *B. pyocyaneus*, the *B. acidi lactici* and rarely the *Bacillus of Friedländer*. Bacterin therapy, especially using autogenous suspensions of the first three named organisms, has in many cases been productive of good results. The initial dose had best be smaller than the average and here the utilization of the opsonic index as a control of treatment will prove serviceable. In not a few cases no material benefit has followed the use of bacterins.

Typhoid Fever.—Inasmuch as the lesions of the intestines stand out conspicuously in the pathology of this disease and are the organs first concerned by the invading microbe, the *B. typhosus*, it may be permissible to discuss its therapy under this heading. In the first place, it will be recalled that not infrequently by bacteriological study the fever will be found to be due to an organism closely allied to the bacillus of Eberth, namely the *B. paratyphosus* A or B or the paracolonic bacilli, hence the advisability of accurate bacteriological investigation if this disease is to be treated biologically. A number of observers have reported favorably as to mortality complications and relapses by bacterial inoculations. In spite of the fact that after the first two or three days a bacteræmia supervenes, enduring for a number of days or weeks, a strong movement is in progress relative to the routine employment of typho-bacterin in

typhoid fever. The argument is not altogether convincing, but the adoption of this mode of therapy is entitled to serious consideration. In ten days or two weeks, after the bacilli are no longer demonstrable in the blood, the expert administration of typhoid bacterin may result advantageously. It would appear that a stock bacterin prepared from an old culture of high antigenic properties is preferable to an autogenous preparation. There exists a danger of the employment of too large dosage. A primary inoculation of 50,000,000 bacilli is proper. The opsonic index will render service in the control of subsequent injections (Fig. 53).

The bacterin treatment of "typhoid carriers," whether the infection lurk in the gall-bladder or urinary tract, has at times resulted brilliantly, at others failed.

Antityphoid inoculation stands out as one of the particular bright lights in bacterial immunization. In the armies of England, France, Germany, Japan, and the United States, adopting prophylactic inoculations, the fall in incidence and mortality rate from typhoid fever has been remarkable (Fig. 57). *Were the practice universal typhoid fever would soon cease to exist.*

In 1918, in the army of the United States, of 90,646 inoculated officers and enlisted men, only three

developed typhoid fever with no fatalities. Antibodies reach their height usually within two weeks after the first inoculation and immunity is alleged to be absolute for one or two years and may endure for many more, although it is advisable to reinoculate after one year if an epidemic threaten or the individual be exposed. The most approved doses for protective inoculation and those adopted by the United States

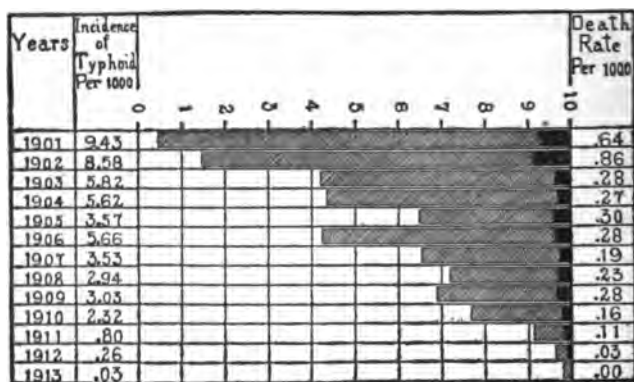


FIG. 57.—Illustrating typhoid-fever rates in United States Army. Shaded columns represent decrease in incidence of typhoid; black areas, the decrease in death-rate.

Army are: First dose 500,000,000; second and third doses 1,000,000,000 each. Intervals of a week to ten days must separate the inoculations. In over 500,000 inoculations in the Army and Navy no bad results have been reported. Clinically, the majority of those inoculated develop fever rarely over 101° F., but at times exceeding even 103° F. Headache, malaise and muscular aches are commonly observed; rarely chills, nausea and vomiting occur. Agglutinins and

opsonins are readily demonstrable (Fig. 58). The time is ripe when it is imperative that physicians, nurses, ward attendants and all those coming in contact with typhoid patients should be immunized. This

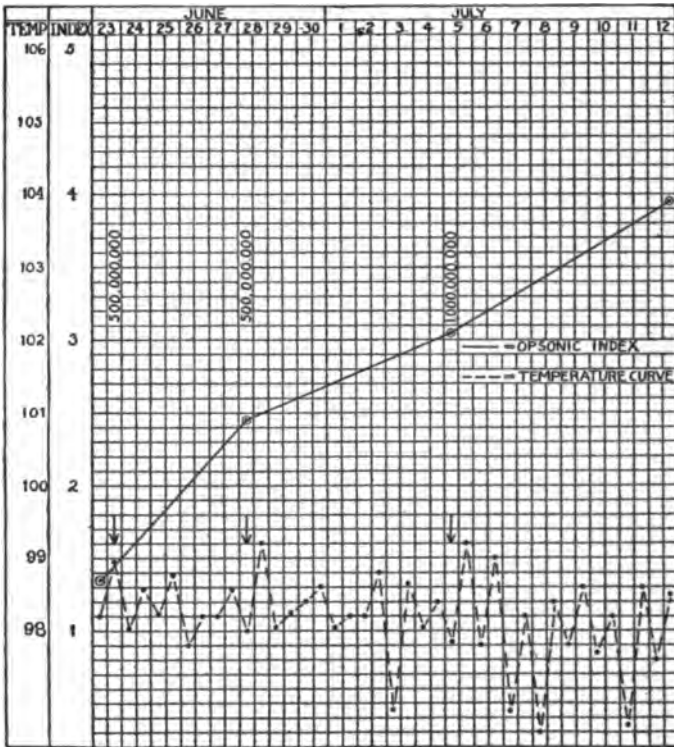


FIG. 58.—Antityphoid inoculation or immunisation. Observe the rapid rise in antibody formation as demonstrated by the opsonic index curve.

rule should include all individuals travelling in and residents of typhoidal districts as well as the members of families and pupils of schools in which the disease has made its appearance.

Dysentery.—Bacterin therapy concerns only the bacillary type of this disease. Closely allied bacilli have been described by Kruse, Shiga, Flexner, Hiss and Strong. Bacterial inoculations, employing mostly the Kruse-Shiga type of organism, have been extensively used in all save the acute gangrenous form of the disease, with the result that the mortality has fallen from 6.8 to 0.9 per cent. It should be noted that in acute cases bacterin therapy is contra-indicated from the fourth to the twenty-first day. During this period Kruse and Shiga's anti-endotoxic serum should be administered in full doses.

Cholera.—Bacterin therapy in this disease is limited entirely to preventive inoculation; it is valueless in a curative capacity. Since the introduction of prophylactic inoculations with the vibrio cholerae, the incidence of the disease in a study of thousands of uninoculated and inoculated cases has fallen from 8.6 to 0.66 per cent. Three immunizing doses of 500, 1000 and 1000 million spirilla respectively are administered at intervals of about ten days.

DISEASES OF CARDIOVASCULAR, LYMPHATIC AND NERVOUS SYSTEMS, ALSO OTHER ACUTE SPECIFIC FEVERS

Bacteræmia, Septicæmia and Pyæmia.—It is our belief, for reasons previously stated under "Contra-indications," that bacterial inoculations, in these con-

ditions, are not only valueless, but harmful, and should the patient recover, he does so not because of, but in spite of, bacterin therapy.

Endocarditis.—The streptococcus of the mitior or viridans, fœcalis or salivarius and pyogenes longus types, the pneumococcus, staphylococcus, gonococcus and *B. influenzae* are held to be the responsible infective bacteria. The results of bacterial inoculations have been far from promising, although a few successes have been reported in the chronic and even in the subacute forms of the disease. Treatment in this disease, if conducted at all, must be with the utmost caution. In the acute form, it is certainly condemnable.

Rheumatic Fever and Articular Rheumatism.—The *Streptococcus rheumaticus* is accepted, in many quarters, to be the causative organism. The results of Buchanan in the acute stage of these affections and those of Veitch in the chronic form, where entirely satisfactory results were obtained in 50 per cent. of cases treated, are worthy of consideration, but as yet have not been duplicated by confirmatory investigation.

Malta Fever.—The *M. melitensis* is the offending bacterium. Although the authors have had no experience, it is claimed on good authority that small doses in the acute stage and larger dosage in the

chronic stage have been followed by undoubted benefit in the treatment of this disease.

Cerebrospinal Meningitis.—It is probable that the value of meningo-bacterin is to prevention as anti-meningitis serum is to cure. As a result of the immunization of over 10,000 persons, none receiving full immunizing dosage developed epidemic spinal-meningitis. Albright states that “meningo-bacterin is of as much value as a prophylactic in meningitis as typho-bacterin is in typhoid fever.”

For purposes of protection, three doses of 500,000, 1,000,000,000 and 1,000,000,000 meningococci respectively are administered at intervals of a week or ten days.

Hydrophobia.—Although the exact nature of the virus of rabies has not been definitely determined, antirabic inoculation as suggested by Pasteur has been extensively practiced and its value universally recognized. In a curative capacity rabies vaccine seems to be ineffective.

After immediate and thorough cauterization of the wound, inflicted by an animal having or suspected of having rabies, the individual should waste no time in undertaking the “Pasteur Treatment.” In the meantime, the animal should not be killed, but securely confined and observed; if rabid, it will die in a few days manifesting symptoms of the disease. If the

animal has been killed, as is commonly the case, the head and attached neck should be sent to a State or municipal laboratory for examination for the characteristic "negri bodies."

Antirabic inoculation consists of a series of daily subcutaneous injections of virus prepared by emulsifying the specially dried spinal cord of rabbits, dead of rabies from a fixed virus. Rabies vaccine is best prepared in a laboratory particularly devoted to that purpose. A number of pharmaceutical firms and rabies institutes, to-day, on request, make daily deliveries in calorix bottles of the amount for administration, making it unnecessary for the patient to patronize an "institute" and rendering it perfectly feasible for the family physician to conduct the treatment at home. All that the practitioner is obliged to do is to note the age of the patient, the date and hour of the bite, its location and extent, if possible whether or not the animal surely had rabies, and furnish these data to the expert or firm about to produce the vaccine. In general the "Pasteur Treatment" comprises twenty-five inoculations administered over a period of three weeks. On the first day three inoculations are given four hours apart, on the second and third days two injections are made at intervals of six hours; on the fourth and succeeding days only one inoculation is administered.

Allusion, however, should be made to the more recent work of Semple and Harris, who have prepared efficient vaccines from the medullas of rabbits, which will keep for long periods and are constantly ready for use, thereby obviating the elaborate methods of Pasteur. Harris in particular has reported on the intraspinal injection in animals of 0.1 milligramme of powder obtained from the pulverized frozen brain and cord of animals killed by a fixed virus. This preparation is dried *in vacuo* over sulphuric acid at a low temperature. It is claimed to produce immunity in a few hours or days, instead of weeks, and can be preserved in sealed tubes indefinitely. Further investigation is necessary to determine whether or not Harris' preparation is to supplant the more familiar "Pasteur Treatment."

MALIGNANT NEOPLASMATA

All attempts to produce an efficient tumor extract or emulsion for the treatment of carcinoma and sarcoma have proved futile. A few years ago Doyen proclaimed the specificity of the *Micrococcus neoformans* in the etiology of cancer. This organism is undoubtedly an accidental invader, and to the best of our knowledge no case of carcinoma has been or will be cured by the agency of neoformans bacterin.

Coley's treatment of sarcoma is worthy of mention.

Coley's fluid, so-called, is a mixed bacterin of the streptococcus of erysipelas and the *Bacillus prodigi-
osus*. It is indicated (1) in all cases of inoperable sarcoma, excepting the melanotic type; (2) for two or three weeks even in operable cases, if there be a chance of saving a limb, that is when the giant-celled type of tumor exists; (3) as a prophylactic against recurrence after operation, and (4) post-operatively, even against carcinomatous recurrence. Apparently, a large number of properly selected cases have been cured by administering daily inoculations, starting with one-quarter minim in the gluteal or pectoral region, and increasing the size of the dose one-quarter minim daily until a reaction is evoked, that is a temperature of 102° to 104° F. The dosage must not be increased and may have to be diminished on the supervision of reaction. It is recommended that the inoculations be made subsequently in the tumor if the patient be not too susceptible. The initial tumor injection should be only one-quarter of the previous inoculation into the gluteal or pectoral region. Coley states that carcinoma is not influenced by the treatment. In the treatment of 480 cases of inoperable sarcoma he reports 47 cases of complete cure, in 28 of which there was no recurrence for three to fifteen years. He asserts that as a prophylactic after opera-

tion the use of the bacterial toxins has reduced the percentage of recurrence from 75 to less than 25 per cent.

YEAST AND SOUR MILK

Brewer's yeast (*Saccharomyces cerevisiæ*) has been a popular remedy in the treatment of furunculosis. In many cases it appears to be without effect and to-day is being supplanted by staphylococco-bacterins. The employment of yeast in tuberculosis and cancer has been a failure.

Metchnikoff observed that the peasants in certain parts of Bulgaria, whose staple diet consisted in the consumption of peculiar soured milks, lived to an advanced old age. A peculiar lactic acid bacillus, the *Bacillus lactis Bulgaricus* or *Bacillus of Massol*, was shown to be the effective organism. Accordingly, the ingestion of sour milk has become a world-wide practice in the hope of avoiding senility, correcting putrefactive intestinal processes and a host of other affections, as infantile diarrhœa and enterocolitis, intestinal indigestion and auto-intoxication, diabetes, rheumatism, gout, arteriosclerosis, etc. Obviously, the results have not measured up to expectations, although benefit seems to have attended the use of artificially prepared soured milk in many cases.

There are many tablets on the market for immediate ingestion or for making buttermilk, purporting

to fulfil Metchnikoff's assertion, but none of these equal in therapeutic value the living liquid cultures of the *B. Bulgaricus*. These cultures are best prepared in tubes as is done by certain pharmaceutical houses. The content of one tube is poured into three table-spoonfuls of sweetened water and drunk, or it may be poured into a glass or half a glass of milk and drunk immediately. Fresh cultures should always be obtained and stored in a refrigerator to maintain activity. They may be taken indefinitely without harmful effect.

APPENDIX

PART A

SERUM TREATMENT OF HEMORRHAGE

NORMAL FRESH SERUM—PRECIPITATED HORSE SERUM— TRANSFUSION OF BLOOD

NORMAL FRESH SERUM

THE most frequent cause of persistent hemorrhage from small vessels is a defect in the coagulation of the blood. Without going into the details of the theory of coagulation of the blood, it is now believed that delayed coagulation is generally due to a deficiency of thrombin or fibrin ferment in the blood-serum. In cases of pathological hemorrhage this deficiency may be supplied by the administration of normal blood-serum, and obstinate and persistent hemorrhage has frequently been checked by this means after all other agencies had failed. This is now recognized as the best form of treatment for persistent capillary hemorrhage whether inherited or acquired, such as that due to hæmophilia, hemorrhage of the new-born, puerperal hemorrhages, pulmonary, intestinal, nasal, and renal hemorrhage, and traumatic hemorrhage. While theoretically human serum should be more effective in treatment, yet Clowes and Busch (*N. Y. Med. Jour.*, Jan. 4, 1913) have shown that, practically, *horse serum* answers the purpose just as well, and is more available. In emergencies, when normal horse serum cannot be quickly obtained, diphtheria antitoxin may be employed with good results.

Administration and Dosage.—The serum is usually administered hypodermically, but may also be given intraven-

ously or by mouth. The dose depends upon the severity and cause of the bleeding, age of the patient, etc. The average adult dose is 20 c.c. hypodermically or 10 c.c. intravenously. If necessary, a larger dose, up to 50 c.c., may be given in two or three days, and this may be succeeded by 100 c.c. a few days later. There is no hard and fast rule as to the size and repetition of doses. One must be guided entirely by the effects. Large doses can be given without injury. The dangers of anaphylaxis may be avoided by repeating the injections at intervals of less than 10 days. In children it is well to begin with a dose of not more than 10 c.c.

Normal horse serum may be applied locally for the control of hemorrhage from wounds. It may be injected through a ureteral catheter in cases of renal hemorrhage.

PRECIPITATED SERUM

Clowes and Busch (*loc. cit.*) first suggested that precipitated horse serum be employed in place of the less convenient fresh serum. To quote from their conclusions: "Blood serum precipitated by means of a suitable mixture of acetone and ether is fully as effective as fresh serum, if not superior to it. Precipitated serum is freely soluble and possesses the advantage over fresh serum of being sterile, always available, and retaining indefinitely its capacity to stimulate coagulability of the blood."

Precipitated horse serum may now be obtained in the form of sterile powder under the name of "Coagulose." This is easily dissolved in sterile water, and is then ready for injection. The powder itself may also be applied to wounds and bleeding surfaces. This powder retains its coagulating properties almost indefinitely, while fresh serum loses its effectiveness in a short time.

TRANSFUSION OF BLOOD

There are many conditions under which transfusion of blood from one individual to another is of great value. Among the commonest of these are: Loss of blood following operations and accidents, severe anæmia from various causes, hemorrhage in typhoid fever, gastric hemorrhage, hemorrhage of the new-born, and postpartum hemorrhage. The introduction of new blood fulfils three functions: (1) It stops the hemorrhage; (2) replaces lost blood; (3) assists in overcoming any infection present by supplying fresh complement and antibodies.

In transfusion the person yielding the blood is known as the *donor*, while the person receiving it is known as the *recipient*. In selecting a donor, it is important that his blood be compatible with that of the recipient. Incompatibility of the two bloods may be manifested in hæmolysis or agglutination of one case by the other, producing grave symptoms or even fatal results. Hæmolysis of the blood of either the donor or the recipient in transfusion is a pathological phenomenon and causes hæmoglobinuria which may be fatal. There is evidence that cases in which hæmolysis occurs *in vitro* will also show hæmolysis *in vivo*, and a preliminary test of the bloods is therefore important in the selection of a donor.

It has not been proved that agglutination of the red blood-cells of the donor by the serum of the recipient, or *vice versa*, gives rise to serious results, and this is therefore not regarded as a contra-indication to the transfusion. In case, however, there should be a considerable range of selection of donors, one should be selected if possible whose blood is not agglutinative.

In every case where practicable, the blood of the donor

should be subjected to the Wassermann test, to avoid the introduction of syphilitic infection into the recipient.

Simple clinical tests for hæmolysis and agglutination have been described by Epstein and Ottenberg (*Arch. Int. Med.*, May, 1909). They have devised a technic in which only very small quantities of blood are required, and which is easy of performance. The blood may be obtained by puncturing the finger as in the case of the Wassermann test. One or two c.c. of blood are allowed to drop into a tube containing an excess of 1 per cent. sodium citrate in normal salt solution. This is centrifuged to wash the red cells, which can be made up to any desired percentage. Another cubic centimetre of the blood is collected, allowed to coagulate, and the serum to separate. In studying hæmolysis, the mixture of serum of the donor and red cells of the recipient, and *vice versa*, must be made within 12 or at most 24 hours of collecting the blood. One volume of corpuscle suspension and one or more volumes of serum are drawn up into a Wright capillary pipette, thoroughly mixed, and incubated in an upright position for two hours. At the end of this time the occurrence of hæmolysis can be easily noted if it has taken place.

It is found that a near blood-relative (sister or brother) of the patient is the most satisfactory donor, with the smallest chances of untoward results. A robust person should of course be selected if possible.

Many methods have been devised for the transmission of blood from one person to another. Some of these are direct, *i.e.*, by the anastomosis of a vessel of the donor with that of the recipient. Other methods are indirect, *i.e.*, by collection of blood from the donor in a suitable vessel, and subsequent introduction of it into the recipient. Brewer and

others have devised paraffin-lined glass tubes for the passage of blood from the vessel of the donor to that of the recipient.

Crile was one of the first to employ the direct method of transfusion extensively, and he unites the radial artery of the donor to a superficial vein in the arm of the recipient by means of a special clamp (*Keen's Surgery*, Vol. V, p. 616). Numerous other instruments have been recommended for this purpose, among others, those of Landon (*Jour. A. M. A.*, Aug. 16, 1913) and McGrath (*Jour. A. M. A.*, Jan. 3, 1914).

The difficulties of blood-vessel anastomosis have led many surgeons to devise various forms of apparatus for removing blood from the donor, preventing it from clotting, and subsequently introducing it into the vein of the recipient. An advantage of indirect transfusion is that the amount of blood utilized can be exactly measured. Kimpton and Brown (*Jour. A. M. A.*, July 12, 1913) have devised a glass cylinder lined with paraffin, into which they withdraw the blood, which is considerably delayed in clotting by the paraffin.

Probably the simplest method of indirect transfusion is that recommended by Dorrance (*Penna. Med. Jour.*, Sept., 1914). The blood is removed from a vein of the donor by means of a 50 c.c. all-glass syringe, in which has been previously placed 10 c.c. of a 10 per cent. solution of sodium citrate to prevent clotting. After withdrawal of a syringe-ful of blood, the syringe is detached, the needle remaining in the vein of the donor in case more blood is required, the lumen being closed with a stilette. The blood mixed with sodium citrate solution is then injected through a needle similarly introduced into a vein of the recipient. Dorrance states that with experience this operation can be

performed so rapidly that no fluid to prevent coagulation need be added.

Two technical procedures can be carried out to guide the operator in the quantity of blood to be transfused. One of these is the estimation of the hæmoglobin percentage of the blood of the recipient from time to time during the operation, and the other is noting the coagulation time of the blood. Many methods have been employed for the determination of the coagulation time, that of Dorrance (*Am. Jour. Med. Sc.*, Oct., 1913) being accurate and practical,

PART B

ORGANOTHERAPY

THYROID GLAND—ADRENAL GLAND—PITUITARY BODY— OVARY—CORPUS LUTEUM—THYMUS GLAND

It has been definitely established that the so-called "ductless glands" furnish secretions that have important functions in the development and metabolism of the body tissues, some of them being essential to life. In the case of certain of these glands, the function of their secretion is known with some degree of accuracy. In the case of others, our knowledge is indefinite or unknown.

It is proposed here to give brief mention to the various derivatives of the ductless glands, with indications for the therapeutic use of those which have proved of value.

THYROID GLAND

For many years it has been well known that atrophy or removal of the thyroid gland causes marked disturbances of nutrition, producing characteristic symptoms grouped under the names *cretinism* (congenital absence of thyroid gland) and *myxædema*. This disturbance is believed to be due to defective tissue oxidation, depending upon the absence from the circulation of the internal secretion of the gland. It has been shown that the thyroid tissue contains more iodine than any other tissue of the body, and that its activity is directly proportional to the amount of iodine present. This iodine is present in the form of iodized proteid (iodothyryn, thyroprotein, etc.). Abnormal activity of the gland-secreting substance produces the symptoms found in the acute stage of exophthalmic goitre or Graves's disease.

For *therapeutic purposes* sheep's thyroid is employed.

Extract of thyroid gland tissue is indicated in cases of undeveloped thyroid (cretinism) and in all conditions where the normal activity of the gland is impaired (myxœdema following atrophy from disease or loss of the gland by operation). The most brilliant results in the whole domain of organotherapy have been seen in thyroid extract feeding in cretinism. In these cases it is customary to begin with a dose of 5 grains of the crude extract three times a day in capsule form. This may be gradually increased according to the effects produced. Thyroid extract has also been employed with success in a great number of other conditions in which defective oxidation is supposed to be a factor, especially obesity, certain skin diseases, and joint affections. It may be used sometimes with good results in the second or atrophic stage of exophthalmic goitre, where there is a functional insufficiency of the thyroid gland, but never in the acute or early stage. Numerous attempts have been made to isolate the active principle of the gland in a more or less pure form, leaving behind the toxic products. Beebe has succeeded in obtaining a thyroprotein or concentrated extract of the thyroid gland which is standardized to contain 0.33 per cent. of iodine. By the use of this standardized product the dosage can be more accurately controlled than in the case of the crude extract. The principal symptoms that the therapeutic limit in thyroid administration is being approached are rapidity of the pulse, overactivity of the sweat glands, and marked loss of weight.

ADRENAL GLAND

The functional activity of the adrenal glands is due to a substance known under various names, such as adrenalin, suprarenin, epinephrin, etc. This substance is a heart stimulant and powerful vasoconstrictor. For therapeutic pur-

poses it is obtained from the ox and sheep, and is also prepared synthetically. It is questionable whether the synthetic product is superior to the natural organ extract. Adrenalin chloride is the preparation usually employed, and is generally supplied in a 1-1000 solution. It may also be obtained in powder or tablet form. Adrenalin, on account of its vasoconstrictor effects, is chiefly employed to control hemorrhage from small vessels, and is useful as a topical application in the treatment of various nasal conditions, such as epistaxis, conditions requiring shrinkage of the turbinates, hay fever, etc. The constricting effects of adrenalin are very transitory, and are usually followed by marked dilatation. Adrenalin is used extensively as an adjuvant to cocaine, novocaine, and other local anæsthetics. The effects of these drugs are considerably enhanced and prolonged by the vasoconstrictor effects of adrenalin. In local anæsthetic mixtures, adrenalin chloride is generally used in the strength of 1-10,000. The cardiac stimulant effects of adrenalin are made use of in the treatment of shock. In this condition it may be given intravenously in normal salt solution in the proportion of 1 drachm of adrenalin chloride to a pint of salt solution.

Suprarenal gland feeding has been employed without benefit in Addison's disease, which is due to atrophy or disease of the adrenals.

PITUITARY BODY

Much knowledge of the pituitary gland or hypophysis and its secretions has been gained within the past few years. Experiments have shown that the anterior lobe of the pituitary body secretes a substance concerned in vital processes, and which is essential to life. The secretion of the posterior lobe or pars nervosa, while not essential to life, contains a

pressor substance, which has a marked action in maintaining continuously high blood-pressure by virtue of its production of peripheral vascular constriction and augmentation of the force of the heart-beat. The effects of this substance upon the circulation are more powerful and lasting than those of suprarenal extract. Pituitary extract in addition produces diuresis by a specific action on the renal epithelium, and is a powerful stimulant to involuntary muscle.

Therapeutic Uses.—Extract of the posterior lobe of the pituitary body is used extensively as a stimulant to uterine contractions in the second stage of labor, and is superior to ergot and other oxytocics. It is very effective in cases of uterine inertia, often rendering unnecessary the use of low forceps, and hastens the course of labor in cases of moderately narrow pelvis. It checks any tendency to severe postpartum hemorrhage. The administration of pituitary extract does not suffice to induce labor or abortion. The dose of the extract is 1 c.c. intramuscularly, and repeated in one hour if necessary. In addition to its uses in obstetrics, posterior lobe extract is administered in various conditions. It is used by Cohen as a cardiovascular support in pneumonia. He employs it hypodermically in doses of 1 c.c. every three or four hours. Pituitary extract is said to be useful in preventing abdominal distention due to temporary paralysis of the bowel after abdominal operations, in surgical shock, and various other conditions.

Contra-indications.—Pituitary extract on account of its marked tendency to increase blood-pressure is to be used with great caution in myocarditis, arteriosclerosis and nephritis. In obstetrical cases it should not be employed in abnormally narrow pelvis, or in threatened rupture of the uterus.

In cases with symptoms of glandular deficiency, such as acromegaly, etc., prolonged feeding with powdered extract

of the whole pituitary gland is indicated, as in these cases probably both lobes of the gland are functionally insufficient. It is stated that moderate doses can be given for a long period without harmful effects.

OVARY

Ovarian substance for therapeutic use is a dry powder prepared from the entire fresh ovary of the hog. It is employed with some success in functional dysmenorrhœa, disturbances of the menopause, etc. In dysmenorrhœa, 2 to 4 grains may be given every four hours until the symptoms are relieved.

CORPUS LUTEUM

The corpus luteum of the ovary produces an internal secretion whose function appears to be to stimulate the menstrual flow and also to sensitize the uterine mucosa for placental formation.

Powdered corpus luteum has been extensively used in various forms of dysmenorrhœa, neurasthenia, functional disorders associated with the natural or artificial menopause, and functional amenorrhœa. It has been found useful in vomiting of pregnancy.

The average dose of corpus luteum is five grains of the dried powder taken three times daily, during meals.

THYMUS GLAND

The thymus gland is a mass of lymphoid tissue situated in the upper anterior part of the thorax. It is well developed at birth, and then under normal conditions gradually disappears. Little is known of the function of the thymus, but it is believed to be concerned in growth and development and to be closely associated in function with the thyroid.

Thymus gland has been used empirically in a number of conditions, among which may be mentioned exophthalmic goitre, rickets, tuberculosis, hæmophilia, marasmus, etc., with varying success. Calf thymus is supplied in desiccated form, and is employed in doses of 2 to 4 grains three times daily.

In addition to those mentioned, preparations from several other organs of animals have been used for therapeutic purposes, such as mammary gland, testicle, parathyroid, parotid gland, spleen, etc., the results of which have not been of sufficient importance to warrant further consideration at this time.

PART C

CHEMOTHERAPY

ADMINISTRATION OF SALVARSAN AND NEOSALVARSAN, INTRAVENOUSLY, INTRAMUSCULARLY AND INTRA- SPINALLY—AUTOSALVARSANIZED AND ARTIFICIALLY SALVARSANIZED SERUM

THE relationship existing between the Wassermann reaction and the treatment of syphilis is so closely interwoven as to warrant a chapter on the subject of chemotherapy in a treatise on serology. It is not our purpose to present a detailed consideration of chemotherapeutics, but simply to refer to this recent branch of scientific medicine, particularly with respect to the administration of salvarsan and neosalvarsan.

It is generally conceded, all things being equal, that immunotherapy, theoretically, is superior and should take precedence to chemotherapy. The reason for this is at once apparent, due to the fact that the former is parasitotropic and not at the same time organotropic. Unfortunately biologic therapeutics has not been applicable to syphilis, consequently the treatment of this disease devolved upon drugs or chemicals, some of which, in spite of the time required to effect cure, have come to be known as "specific," for example mercury.

HISTORY OF SALVARSAN AND NEOSALVARSAN

Ehrlich, attracted by the alleged value of atoxyl in trypanosomiasis, and mindful of the possibility of increasing the antiseptic effect of drugs, at the same time diminish-

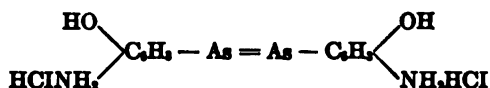
ing the toxic, by the introduction of the halogen group into the benzene ring, conceived the idea that arsenic in certain combinations could be so compounded that its administration in a sufficiently large dose would not be injurious or toxic to the organism and would at the same time destroy all spirochætæ, sterilizing the system. Repeated trials enabled Ehrlich and his collaborators to produce a synthetic drug, No. 606 in the experimental series, later styled "salvarsan," the use of which was claimed to be a "therapia sterilisans magna" for the cure of syphilis. Subsequently, further refinement led to the production of neosalvarsan or No. 914, apparently less toxic but likewise less efficient.

Be it understood that the treatment of syphilis by arsenical preparations is not by any means a new idea. Syphilologists for decades have recognized the benefit to patients when arsenic supplemented or alternated mercury and iodine, but owing to the toxicity of the trioxide of arsenic, it has been necessary to administer it in minimal dosage. Consequently, during recent years other forms of arsenic, less toxic and of higher drug content, have been synthesized, recommended and utilized. Among these arsenical preparations may be mentioned the arylarsonates (soamin and orsudan), atoxyl, arsacetin, arsenophenylglycin paramidophenylarsenoxide and sodium cacodylate. Some of these have fallen into almost complete disrepute owing to gastro-intestinal disturbances, nephritis and neurological manifestations, including blindness, occasioned by their administration, and all have failed to measure up to the virtue of salvarsan and neosalvarsan.

Valuable and important as Ehrlich's discovery has already proved itself to be in the treatment of syphilis, admittedly the sheet-anchor in the pharmacology of that disease, it is extremely doubtful if salvarsan will prove to be

the universal, omnipotent, all-sufficient panacea for lues originally claimed by its advocates, although the authors have repeatedly observed, in the primary stage of the disease before the advent of a positive Wassermann, a single intensive dose of salvarsan suffice to effect a cure, thereby fulfilling the dictum of Ehrlich. Possibly the cure not attained by a single dose may be accomplished by repeated injections or a combination with mercury and iodine. This much only is assured at present, that in the great majority of patients the immediate effect of salvarsan properly administered is a symptomatic cure; concerning the remote results, additional time must elapse before definite conclusions can be drawn.

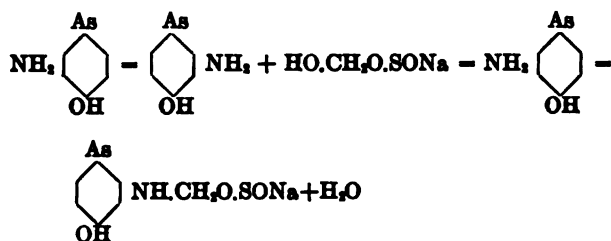
Chemico-physical Properties.—The Ehrlich-Bertheim-Hata arsenical preparation No. 606, synthetically known as dioxy-diamido-arseno-benzene-dihydrochloride, may be represented by the following structural formula:



It is commercially known in the market at present as "salvarsan," and appears as a light yellowish powder, markedly acid in reaction and very unstable on exposure to the atmosphere. For these reasons the drug must be neutralized and prepared in solution strictly fresh each time before its administration.

Ehrlich observed that formaldehyde-sulphoxylates of sodium possess the property of restraining for a time the auto-oxidation of solutions of dioxy-diamido-arseno-benzene, and by the interaction of the two evolved dioxy-diamido-arseno-benzene-monomethane-sulphinate of sodium or No. 914, com-

mercially known as "neosalvarsan." It may be represented by the following structural formula:



Neosalvarsan appears as an orange-yellow powder of peculiar odor, dissolving very easily in cold water with completely neutral reaction.

Neosalvarsan is even more unstable than salvarsan, and although slightly less trouble to prepare for administration, requires even greater precaution as to freshness and temperature of solution, owing to its greater proclivity to oxidation and the formation of products of a high degree of toxicity. Unfortunately, its ease of solution in water, at once in a neutral state ready for administration, and better toleration by patients, does not outweigh the greater effectiveness and spirochæticidal power of salvarsan, the injection of which with proper care and technic is attended with a minimum degree of reaction.

Indications.—Salvarsan or neosalvarsan is indicated in all stages of syphilis, and should be the first thought in treatment, provided no contra-indications exist. Indeed, in the early primary stage cure may often be obtained by the administration of salvarsan alone, particularly before the appearance of a positive Wassermann reaction. It seems especially effective in those cases where mercury and iodine have failed. In cerebrospinal syphilis the effects are variable. For instance in gummatous meningitis, whether diffuse

or localized, with expressions as epilepsy, monoplegia, etc., remarkable results are observed. In *tabes dorsalis*, particularly if the intravenous administration of the drug be supplemented with intraspinal injection of autosalvarsanized or artificially salvarsanized serum, there is reasonable hope of arresting the disease if attacked in its infancy. In paresis and encephalitis the authors have never observed noteworthy benefit, the diseases exhibiting continuous retrogression. The drug is of great value in hereditary lues and in pregnant syphilitics.

The *provocative* employment of salvarsan and neosalvarsan is a matter of considerable importance. The arsenical preparations similar to mercury may be utilized to provoke a positive Wassermann reaction in a syphilitic patient otherwise exhibiting a negative serological reaction. Thus in patients who have previously received antisyphilitic treatment, or in whom the clinical evidence for syphilis is strong and the ordinary Wassermann reaction has resulted negatively, the so-called provocative dose of salvarsan, neosalvarsan or mercury should be employed, and the blood-serum again tested at the end of twenty-four to forty-eight hours. It has been our practice to use 0.1 gramme of salvarsan or 0.15 gramme of neosalvarsan, administered intravenously, for this purpose. The explanations offered for the employment of arsenical and mercurial preparations in this rôle are that the positive serological reaction is due to the liberation of endotoxins from the killed spirochætæ or to the stimulation of the latent spirochætæ by a dose of the drug insufficient to effect a complete destruction. The phenomenon or biochemical reaction is doubtless closely related to the Herxheimer reaction.

In *non-syphilitic diseases*, salvarsan enjoys an extensive employment. Indeed in relapsing fever, tertian malaria,

frambœsia (yaws or pian), filariasis and Vincent's angina, salvarsan appears to be equally as specific as in syphilis. It may be employed in nervous disorders in which arsenical medication is indicated and exerts either a beneficial or curative effect, according to Best, in acanthosis nigricans, ulcus tropicum or phagedœnicum, variola, verrucæ planæ, Sydenham's chorea, scurvy, dermatitis herpetiformis, quartan and tropical malaria. Thus in many diseases the tonic, stimulative and alterative action of arsenic is linked with the germicidal effect of the drug. Good or indifferent results have been reported in Aleppo boil (Oriental sore), anæmia, keratosis follicularis, leprosy, lichen planus, lupus vulgaris, mycosis fungoides, pellagra, pityriasis rubra, tuberculosis and experimental tick fever.

In chancroid, bilharziasis, Hodgkin's disease, psoriasis, scarlet-fever, trichinosis, sarcoma, carcinoma and trypanosomiasis, the drug appears to be without appreciable effect. In explanation of the last it is alleged that the trypanosomes are more susceptible of being rendered arsenic-fast than are the spirochætæ.

In veterinary medicine salvarsan has rendered signal service and a specific effect, particularly in pleuropneumonia of horses and African glanders (lymphangitis epizootica).

Acquired Resistance of Spirochætæ to Salvarsan.—It is generally known that trypanosomes possess the power of adapting themselves to circumstances, for instance preservation against injurious influences. In other words, they may become immune to their own antibodies or chemicals directed against them, as arsenical preparations. This resistive property then becomes a characteristic of the organism and may be transmitted from generation to generation. Although this characteristic has never been demonstrated to obtain for the spirochætæ pallida, it is presumed from anal-

ogy and clinical experience that *spirochætæ pallida* may also exhibit *drug-fast* properties. Thus are explained the failures of mercury to influence syphilis after the first few months in many cases. In like manner, the *spirochætæ* may become tolerant or resistant to the influence of arsenic when administered in salvarsan and neosalvarsan. It would appear that early, small or sublethal doses of these drugs may be responsible for the production of *arsenic-fast* or immune *spirochætæ*. Consequently, the inference is strong that the treatment of syphilis should be intensive from the start, thereby avoiding relapses, significant of immunity on the part of the *spirochætæ*. This is best accomplished by repeated full-sized intravenous injections of salvarsan, the number being controlled by the stage of the disease and the Wassermann reaction.

Contra-indications and Precautions.—Salvarsan is contra-indicated in advanced degenerative diseases of the central nervous system, in severe non-syphilitic retinal and optic disease, in marked disturbances of the cardiovascular system, as acute endocarditis, myocarditis, with or without nephritis, extensive degeneration of the blood-vessels, and angina pectoris, in any form of non-luetic nephritis, in diabetes, in aneurism independent of lues, in pronounced foetid bronchitis and pulmonary tuberculosis, and in persons manifesting an idiosyncrasy for arsenic. Chronic valvular heart disease, syphilitic endarteritis, aneurism and endocarditis are not contra-indications. In cases of malnutrition, cachexia and infantile congenital syphilis great care as to dosage must be exercised. In incipient tabes, early paralysis and epilepsy of syphilitic origin, salvarsan can be employed successfully only when administered early.

Certain precautions are imperative and must be observed

for the proper, safe and best administration of salvarsan and neosalvarsan. They are as follows:

1. An exact technic.

2. Observance of the integrity of the hermetically sealed ampoule and the normal physical characteristics of the contained drug.

3. Immediate and fresh preparation of the solution just prior to administration, avoiding improper degree of temperature.

4. Selection of the appropriate dose for the individual case.

5. Previous examination of the patient to receive the drug, particularly investigation of the renal, cardiovascular and nervous systems.

6. Assurance that any previous administration of mercury has not caused kidney irritation. Wechselmann, who has given over 30,000 doses of salvarsan, cautions against the combined use of salvarsan and heavy mercurial treatment, and protests vigorously against employment of salvarsan after a course of mercurial treatment.

7. Careful notation after injection of a patient of reactionary phenomena, with respect to repeated injections, both as to time and dose; the most important consideration is obviously the possibility of kidney irritation.

8. Caution as to the resumption of vocational activities by the patient too soon after the administration of salvarsan and neosalvarsan.

Dosage.—Michaelis has stated the average dose of salvarsan to be 0.01 gramme to every kilogramme of body weight. Salvarsan is marketed in original packages of hermetically sealed ampoules containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 1.0, 2.0 and 3.0 grammes; of these the average adult dose is 0.6 gramme, which at times may be advantageously dimin-

ished by 0.1 gramme for women. In children the dosage should be proportionate to the age. Syphilitic new-born infants should receive from 0.02 to 0.1 gramme of the drug.

Neosalvarsan, although less toxic, is unfortunately also less effective than its predecessor, salvarsan. The average adult dose is 0.9 gramme, corresponding in arsenical content to 0.6 gramme of salvarsan. Commercially it is marketed in ampoules with the following proportionate dosage:

Dose		Dose
0.15 gramme neosalvarsan	=	0.1 gramme salvarsan
0.3 " "	=	0.3 " "
0.45 " "	=	0.3 " "
0.6 " "	=	0.4 " "
0.75 " "	=	0.5 " "
0.9 " "	=	0.6 " "
1.5 grammes "	=	1.0 " "
3.0 " "	=	2.0 grammes "
4.5 " "	=	3.0 " "

The last three doses are for veterinary purposes only and have no place in human therapeutics.

The repetition of the dose is dependent upon the method of administration (intravenous or intramuscular), the organic condition of the patient and his susceptibility to reactionary phenomena. Usually when injected intravenously, the doses are repeated in five to ten days in the absence of reactionary contra-indications. In some cases weekly administration of 0.6 gramme of salvarsan intravenously for several weeks has been unproductive of harmful effect. The policy should be, particularly in early syphilis, to produce an intensive or specific effect by repeated intravenous injections until a negative Wassermann reaction is obtained. It is seldom that less than three administrations are necessary

and often a dozen or more injections are required to render the Wassermann reaction negative. Obviously, with feeble or prostrated patients of those manifesting a cardiovascular or central nervous system lesion, the initial dose, at least, must be smaller than the average.

When a tonic and alterative effect, in conjunction with specific action, is desired, full doses of the drugs at intervals of several weeks, may be administered intramuscularly, or, if merely the tonic, alterative effect of the drugs is desired, small and frequently repeated intramuscular injections may be desirable. An intramuscular method of administration is that by fractional doses. 0.1 to 0.2 gramme of salvarsan suspended in oil is injected every other day until a total of 1.2 grammes has been given to the patient. This mode of therapy applies more particularly to the treatment of non-syphilitic diseases. Small intramuscular injections, lacking sufficient remedial action, may invite relapses, consequently it is advisable either to precede or supplement the intramuscular injection by a full dose of the drug intravenously. In all stages of syphilis, with the possible exception of the early primary, it is imperative to supplement salvarsan with mercury or mixed treatment.

Methods of Preparation and Administration.—Time and experience have evolved four methods¹ for the employment of salvarsan and neosalvarsan: the *subcutaneous*, the *intramuscular*, the *intravenous* and the *intraspinal* or *intra- or subdural*.

Following the successful original experiments on animals by Hata, Hoppe, in the Clinic of Professor Konrad Alt in Uchtspringe, on the suggestion of Ehrlich, in September,

¹ The administration of arseno-benzol by mouth is unworthy of consideration. The same objection obtains respecting its use as a dusting powder for the chancre on account of cost if for no other reason.

1909, was the first to try salvarsan on the human subject. The preparation used was an alkaline solution of the disodium salt. Shortly afterward Michaelis, Wechselsmann and Lange devised the method of neutral suspension injections and practiced the same subcutaneously and intravenously in thousands of cases. Other notable methods that have been advised are Junkemann's and Lesser's modification of Alt and Hoppe's alkaline method and Kromayer's suspension of salvarsan in paraffin. About this time Schreiber, associated with Hoppe, described the method by intravenous administration. Wechselsmann appears to be the first to have employed salvarsan by intraspinal injection. Subsequently, Marie and Levaditi likewise injected neosalvarsan directly into the spinal canal. While the technic of direct intraspinal injections of salvarsan and neosalvarsan was being perfected, even before the use of hypertonic solutions of neosalvarsan as practiced by Ravaut and Wile, Swift and Ellis recommended the intraspinal injection of auto-salvarsanized serum. This method is less irritating to the nervous system and less dangerous and consequently is the most popular form of subdural arsenic medication. Recently Fordyce has recommended adding minute quantities of salvarsan or neosalvarsan to the auto-salvarsanized serum prior to intraspinal injection.

Subcutaneous Administration.—The subcutaneous administration of salvarsan or neosalvarsan, either the injection of the full therapeutic dose at once or repeated injections of fractional doses over a period of time, is practically an obsolete method. This has been due to great and long persistence of pain, induration, non-absorption of the chemical and necrosis of the skin.

Intramuscular Administration.—The intramuscular injection is superior to the subcutaneous inasmuch as the above

noted objections are not so marked, although not infrequently encountered, subsequent pain and induration being the most objectionable. Occasionally the indurated area may undergo liquefactive necrosis, resulting in sinus formation.

Owing to the fact that all the arsenic, detectable in excreta, is eliminated in three to six days after intravenous administration, while it persists for a much longer period following intramuscular injection, it has been recommended and is practiced by some clinicians to supplement the intravenous in the course of a week by intramuscular injections. The rationale of this *modus operandi* is not without reason and is deserving of consideration. It can be explained on the assumption that by the intravenous injection the drug is at once conveyed to the remotest parts of the body, where it is needed to destroy the spirochæta, thus producing a so-called "intensive" action in contradistinction to the "permanent" action when the drug is exhibited locally and an intramuscular depot is established.

The *technic of intramuscular injections* is somewhat variable. Three solutions have been extensively employed: (1) the alkaline solution, (2) the oily suspension and (3) the neutral suspension. Of these the alkaline solution method is to-day probably most commonly utilized. The preparation of the solution may be similar to that described under Intravenous Administration, differing only in that for intramuscular injection the drug is dissolved and injected in 20 c.c. of fluid, one-half the quantity being injected on either side.² A common procedure for the preparation of the alkaline solu-

² For the intramuscular injection of neosalvarsan a five per cent. solution is employed, since one gramme of the drug dissolved in twenty-two cubic centimetres of water makes an isotonic solution. Therefore, for each 0.15 gramme of neosalvarsan about three cubic centimetres of freshly distilled water should be added.

tion is to triturate 0.6 gramme of salvarsan in a sterile mortar with twenty-three drops of fifteen per cent. sodium hydroxide solution, then to dilute with distilled water to the desired volume.

More recently intramuscular injections have been extensively made with simple suspensions of salvarsan and neo-salvarsan in oils, 1:10 dilution, such as sesame, olive sweet almond and paraffin. Certain pharmaceutical laboratories market the neutral, water-free solidified fat suspensions, liquid at body temperature, in sterile ampoules, requiring merely a syringe for their administration.

The neutral suspension, at one time largely used, both intramuscularly and subcutaneously, at present enjoys a very restricted employment. It is prepared by triturating carefully 0.5 or 0.6 gramme of salvarsan with eight or ten drops of fifteen per cent. caustic soda solution in a sterile porcelain dish. To this is added at first drop by drop with constant trituration the required quantity of sterile water (5 to 10 c.c.). The fine suspension thus produced is tested *exactissime* with litmus paper for neutral reaction, and a drop of the soda solution or hydrochloric acid added in accordance with the reaction.

The upper outer quadrant of the gluteal region is the most desirable locality for intramuscular injection, by virtue of comparative freedom from nerves and blood-vessels (Fig. 59). The vicinity of the sciatic nerve must be carefully avoided. The injection should be given deeply and slowly, thereby obviating hemorrhage and rupture of the muscular tissue. The skin is conveniently disinfected at the site of injection by three to five per cent. tincture of iodine. After injection the fluid is distributed as widely as possible by careful massage, and the needle puncture sealed with collodion. In sensitive patients the area to be injected may be



FIG. 59.—Site for deep intramuscular injection. The needle is plunged inward and downward at a point selected anywhere on the line *CD*, which indicates a region in the upper outer quadrant of the buttock free of large vessels and nerves. The line *CD* lies two finger breadths below the iliac crest; the point *C* located on the vertical line *AB* erected midway between the tuberosity of the ischium and the great trochanter of the femur.

anæsthetized, preliminarily, by injecting through the needle, *in situ*, two cubic centimetres of a one per cent. or five cubic centimetres of a one-half per cent. novocain solution. Post-injectional pains or reactive painful infiltrations may be combated locally with hydrotherapeutic measures, as the hot-water bottle, hot compresses, etc., and internally the administration of pyramidon has proved very effectual. Patients had best remain in bed for some time after the injection.

Unfortunately, no patient who has ever received salvarsan or neosalvarsan intramuscularly desires it a second time by that method, and any patient who has had the drug administered both intravenously and intramuscularly invariably prefers the former because of the lesser pain. Certainly there are occasions when, by force of circumstances, it becomes necessary or expedient to use the intramuscular method, but these exceptions must be few in number.

Intravenous Administration.—The intravenous administration of salvarsan and neosalvarsan in dilute solution is the best method and the mode of therapy of the future. The consensus of opinion among those of widest experience is that salvarsan is more effective than neosalvarsan. A routine procedure with the authors is to commence treatment with neosalvarsan, which, if well tolerated, is supplemented by salvarsan. We firmly believe in the intensive form of treatment, with proper observance of precautions and contra-indications, attempting thereby to eradicate the disease; particularly is this true in the primary stage of syphilis, when the indication for the intravenous administration of arsено-benzol is just as acute as the scalpel in a well-defined case of appendicitis. In the early days of the chancre, especially before a positive Wassermann is obtainable, it is possible to cure syphilis by one or more injections of salvarsan alone; in the late primary usually, and throughout

the secondary, latent, tertiary, nervous and hereditary forms of the disease, mercury or mixed treatment must supplement the administration of arseno-benzol. We seldom administer less than three intravenous injections, usually at intervals of a week. Then, after a respite of three weeks, the blood is tested by the Wassermann reaction. If the result still be positive, three more injections are given and so on until in a few cases a dozen or more doses have been administered. As soon as a negative Wassermann is obtained the patient is subjected to a vigorous course of mercurial or mixed treatment for three months to a year. Treatment is then suspended and the further conduct of the case may depend upon Wassermann reactions of the blood taken at three-month intervals, associated in certain cases with the Wassermann reaction and cytological examination of the spinal fluid. Although no absolute law may be promulgated, no case should be pronounced cured until consecutive negative Wassermann tests are obtained for a period of at least two years after the suspension of all treatment. (See technic described in Chapter XII.)

Preparation of Patient.—After determining the organic fitness of the candidate, the patient to receive an intravenous injection of salvarsan or neosalvarsan should receive a cathartic the night before the injection of the drug. He should take no food at the meal immediately preceding the introduction of the remedy, but may be allowed limited quantities of liquid. He should be placed in the supine position on a table, the arm (preferably the left in right-handed individuals) slightly abducted, the forearm supinated and resting on a support, the elbow very slightly flexed, as shown in Fig. 60. While an assistant sterilizes the region of the elbow, with soap and water, alcohol and a solution of bichloride, the operator, always with the strictest observance



FIG. 60.—Position of patient for intravenous injection of salvarsan. Note the easily applied and released rubber dam tourniquet shown in the corner sketch; also the manner of fixing the skin overlying the vein with the thumb as the needle is introduced into the vein.



FIG. 61.—Showing various reagents, solutions, glass vessels and apparatus ready for preparation of salvarsan or neosalvarsan.

FIG. 62.—Thomas' salvarsan and neosalvarsan outfit.

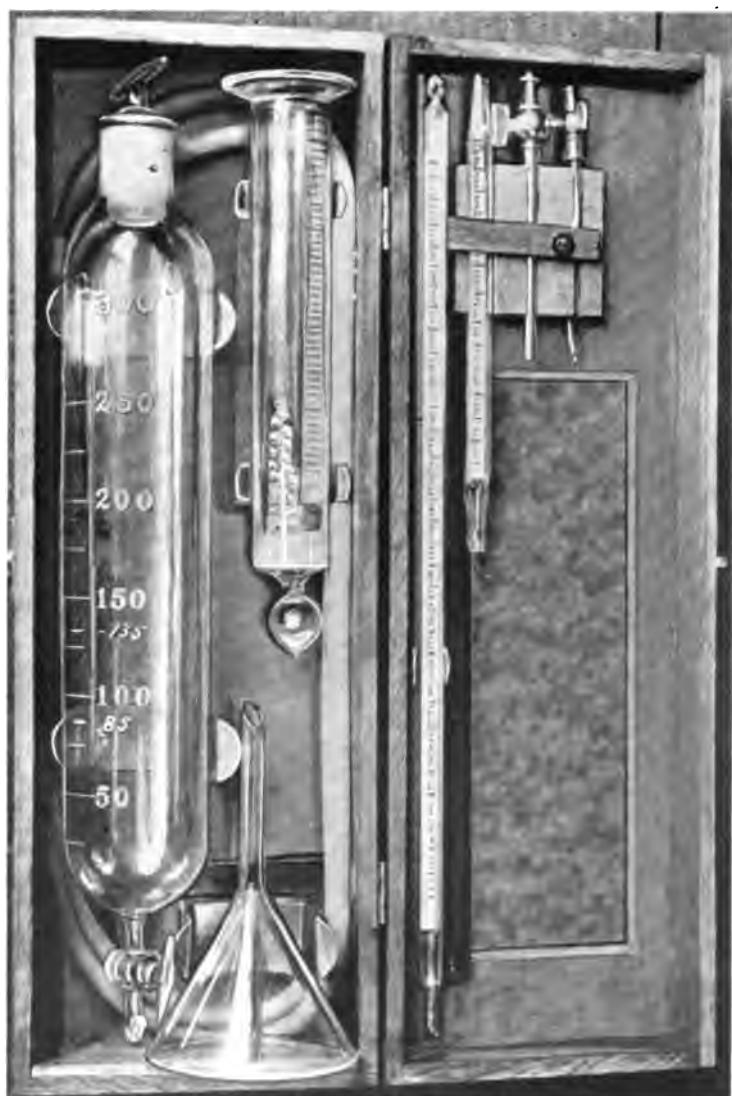




FIG. 63.—Water still as used in authors' offices. The retort and condenser are made of Jena glass to insure freedom from chemical impurities during distillation.

of the chemical and aseptic precautions, prepares the solution for intravenous administration.

Preparation of Salvarsan Solution.—In Fig. 61 are shown the various reagents, solutions, glass vessels and apparatus useful for the preparation and administration of salvarsan intravenously. All apparatus, as described in the outfit (Fig. 62), here recommended, including thermometers, burette, etc., may be sterilized by boiling. The ampoule of the drug is best antiseptized by submersion in a cold solution of bichloride or formalin, after noting that it is intact and its content in no way oxidized. The water and saline solution employed for solution and dilution of the salvarsan must be sterile and freshly distilled. Chemically pure sodium chloride must be used in making the saline solution. The common laboratory or commercial distilled water is usually not sterile. Clinical experience has demonstrated that reactions following the administration of salvarsan are more common when spring, tap or stale water is used than when freshly distilled water is the diluent. Whether or not this increased toxicity is due to the interaction of the endotoxins of the contained flora and arseno-benzol or is referable to other causes, it is advisable to employ freshly distilled water. This may be readily obtained in sufficient quantities by an apparatus (Fig. 63) capable of being installed in the physician's office. Sterilization may then be assured or completed in an Arnold sterilizer for one-half hour. *The use of sterile freshly distilled water and chemically pure salt solution and the preparation of the salvarsan solution immediately before intravenous injection are imperative conditions.*

Twenty to forty cubic centimetres of warm (110° to 120° F.) sterile freshly distilled water, (W, Fig. 61) are placed in the graduated glass stoppered cylinder or mixer (M) containing a dozen or more small glass balls. The

neck of the ampoule (A) containing salvarsan (usually 0.6 gramme) is nicked with the small file accompanying the commercial package and the neck easily broken. The content is emptied into the mixing cylinder and the substance entirely dissolved by shaking, producing a clear light-yellowish solution of a strongly acid reaction. In order to fit this solution for human administration it must be neutralized. Accordingly 1.14 cubic centimetres or approximately 23 minims of a freshly prepared fifteen per cent. solution of purified sodium hydroxide (C) are added. For this purpose a medicine dropper (D) is convenient. The following table, dependent upon the quantity of salvarsan utilized, may be of service:

Salvarsan		15 per cent. Solution of Sodium Hydroxide			
0.6 gramme requires		1.14 c.c. or approximately 23 to 24 minims.			
0.5	" "	0.95	" "	"	19 " 20 "
0.4	" "	0.76	" "	"	15 " 16 "
0.3	" "	0.57	" "	"	19 "
0.2	" "	0.38	" "	"	8 "
0.1	" "	0.19	" "	"	4 "

The addition of the caustic soda, which should be in one quantity and not added slowly drop by drop, produces a heavy whitish yellow precipitate, redissolved in excess on shaking; if necessary another drop or two of the caustic soda solution may be added. At this juncture the solution should be neutral and perfectly clear, resuming its original light yellowish coloration. It is advisable now to add just one more drop of the soda solution, which will render the solution very faintly alkaline. Slight turbidity or cloudiness of the solution is an indication that insufficient sodium hydroxide has been added. If in doubt the reaction should be tested with litmus paper (L) and the neutrality, weak alkalinity or acidity of the solution definitely determined. If decidedly alkaline, a drop or two of the dilute hydrochloric acid (H)

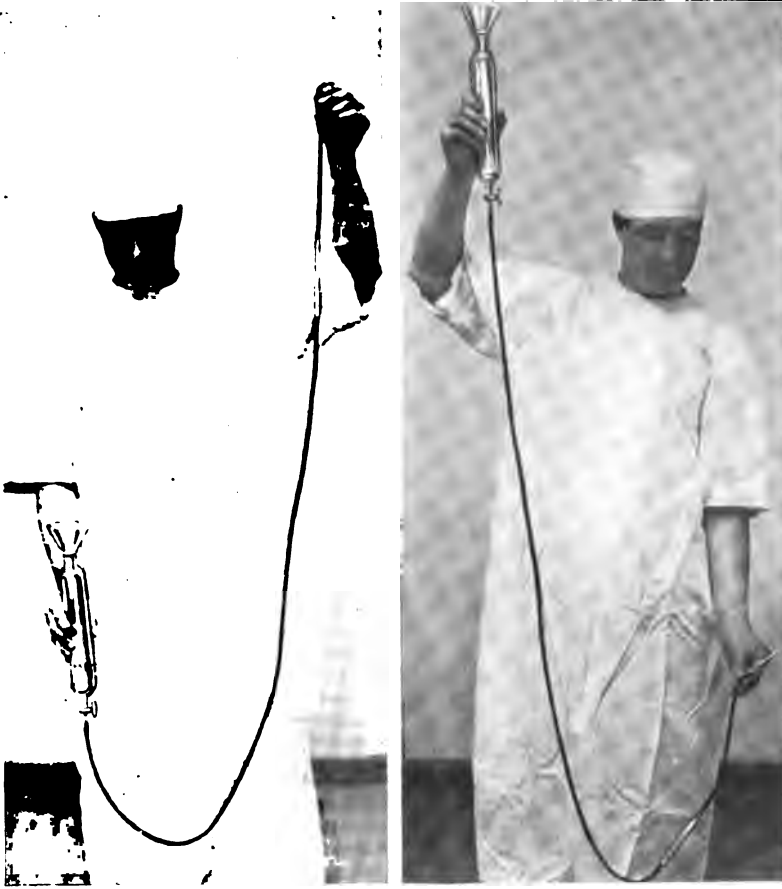


FIG. 64.—Illustrating method of eliminating air from tubing. The burette and needle are alternately elevated and lowered, and when the tubing is entirely free of all air bubbles, the two stop-cocks, one in the needle and the other in the burette, are turned off, leaving the tubing and needle filled with normal saline solution.

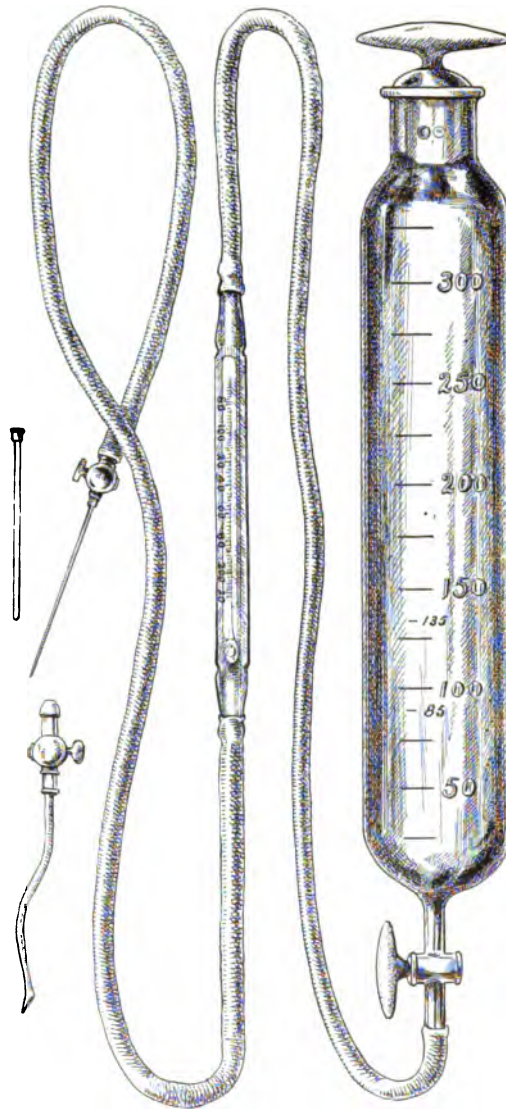


FIG. 65.—Thomas' salvarsan and neosalvarsan burette. The length of the rubber tubing between the needle and transfusion thermometer should not be more than eight inches, and that between the thermometer and burette proportionately increased in length.

must be added. The additions of caustic soda or hydrochloric acid can best be controlled by taking them with pipettes or droppers (D) from the medicine glasses (G). The rubber tubing incorporating the transfusion thermometer (T) and fitted with the platinum-iridium needle (P) or the blunt infusion needle (N), rarely necessary, is adjusted to the burette, which with the tubing is thoroughly rinsed out and the tubing and needle filled with warm normal salt solution (S), the tubing being freed of air by alternately elevating and lowering the burette and needle, as shown in Fig. 64. The weakly alkaline solution of salvarsan is then filtered through sterile cotton contained in the funnel (F) into the graduated burette (Fig. 65). Sufficient warm (110° to 120° F.) sterile 0.5 per cent. saline solution made from freshly distilled water is then passed through the cotton filter, filling the burette to the 300 cubic centimetre mark.³ This is the proper dilution for 0.6 gramme salvarsan. The quantity is diminished by 50 cubic centimetres for each 0.1 gramme less of salvarsan.

With the patient supine and the arm moderately abducted and forearm supinated, the region of the elbow is antiseptitized with soap and water, alcohol and bichloride or a three

³ In view of the recommendation that the preferable diluting fluid for salvarsan is 0.5 per cent. saline, while for neosalvarsan it is 0.4 per cent. salt solution, the authors have found it practicable and convenient from the standpoint of preparation of solutions to utilise two standard solutions—0.9 per cent. chemically pure sodium chloride solution and freshly distilled water. From these any desired strength saline solution can be readily prepared. For instance, in diluting the salvarsan solution, distilled water is added until the quantity in the burette reaches 135 cubic centimetres, then 0.9 per cent. salt solution is added until the amount equals 300 cubic centimetres. In the case of the full-sized dose of neosalvarsan, the dilution is made first with distilled water to the 85 cubic centimetre level, then 0.9 per cent. salt solution is added until the quantity in the burette stands at 150 cubic centimetres.

per cent. tincture of iodine, and a tourniquet of rubber dam (R) is placed around the arm, sufficiently taut to render the superficial veins about the elbow prominent, care being exercised not to obliterate the radial pulse (Fig. 60). The median cephalic or basilic vein is preferable to a vein exactly in the cubital fossa, because, should inflammation in or about the vein supervene, the patient will experience less discomfort on flexion and extension of the elbow. The burette containing the required quantity of salvarsan solution is given to an assistant to hold or it is suspended on a stand adapted to the purpose (Fig. 60). At this point it is usually possible to plunge the sharp pointed platino-iridium obliquely through the skin into the vein, holding the needle with the eye directed downward, at the same time fixing the skin in the axis of the vein about two inches distant from the site of puncture with the index finger or thumb of the hand not holding the needle. As the needle is slowly and steadily advanced at an angle of about thirty degrees, always keeping it at the plane of the axis of the vein, it will usually be possible to see a slight dimpling of the skin overlying the vein at the point where the needle will pierce the vein. Just as the sensation, imparted to the fingers, of decreased resistance is experienced, as the tip of the needle enters the lumen of the vein, the skin dimple likewise disappears. Rarely, it will be necessary in the case of young individuals, some women and those exhibiting a thick layer of panniculus adiposis, when the veins are extremely small or obscured by fat, to infiltrate the skin with a few drops of a one per cent. novocain solution, and then make a cutaneous incision one-half to three-quarters of an inch in length, exposing the vein. The vein may or may not be lifted from its sheath, but in any event it is then possible to introduce the needle without difficulty, using preferably the sharp-pointed one or occasionally the well-

known dull-tipped intravenous variety (N, Fig. 61). If it be necessary to cut the skin, the wound must be closed by a suture.

Great care should be exercised to see that the tip of the needle lies within the vein, because the escape of the salvarsan solution into the subcutaneous and perivascular tissues is productive of great pain, cellular infiltration and prolonged induration and nullifies the advantage of the intravenous over the intramuscular method of administration. For this reason, it is well to have the needle and rubber tubing filled with normal salt solution, which will not produce irritation should it be allowed to escape into the subcutaneous areolar tissue. If, by accident, a quantity of salvarsan solution should be allowed to infiltrate the perivascular tissues, it is advisable to incise the overlying skin immediately, using an anæsthetic of novocain or eucaine, wash out the salvarsan with normal saline, and then to puncture the vein directly. So soon as the vein has been entered, the tourniquet is loosened, the cocks of the burette and needle turned on, and the solution of salvarsan permitted to flow very slowly from the burette. The temperature of the solution just before entering the vein can be read from the thermometer and should be blood heat. The introduction of the solution should not take less than 10 minutes. Just before the salvarsan solution in the burette falls to the level of the stop-cock, it should be turned off, or the rubber tubing temporarily compressed and about twenty-five cubic centimetres more of salt solution filtered into the burette, when the compression is released or the stop-cock again turned on. This procedure permits of the patient receiving the full amount of salvarsan and at the same time suffices to wash the vein free of salvarsan, thereby avoiding the possibility of phle-

bitis, etc. A dry sterile gauze dressing following the removal of the needle completes the operation.

A too rapid introduction of the solution naturally might cause dilatation of the right heart with its attendant results. Too much care cannot be exercised to exclude air bubbles from the apparatus, although the danger from air embolism is not great—incomparable in comparison with the advent of air through the great veins of the neck, where a negative pressure exists—it is nevertheless a serious concern. Similarly, the filtration of all solution entering the vein is a matter of importance, lest solid particles of matter, as glass, acting as emboli, might exert a harmful effect. Thrombosis is an accident alleged to have occurred. This must supervene only as a result of faulty technic or in the presence of an infection and emphasizes the necessity of thorough sterilization of all apparatus and material employed. It is assuredly more likely to ensue when the vein is exposed, due to atmospheric influence on the blood and to metabolic changes in the vein wall. In an experience covering several thousand administrations, it is a complication which we have never encountered.

Preparation of Neosalvarsan Solution.—The solution of neosalvarsan is slightly less trouble to prepare than salvarsan, since when dissolved it is at once neutral and needs no addition of alkali to counteract the acidity. It is far more unstable than salvarsan, consequently must be used immediately after preparation. Moreover, the solution must never be heated. In fact, it is emphatically stipulated that the temperature of the freshly distilled water or salt solution must be that of the room, that is, 68° to 72° F. This had best be determined by an appropriate thermometer (T, Fig. 61). Undue shaking is also to be avoided.

The content of the full adult dose, 0.9 gramme, is dissolved

in 15 to 20 cubic centimetres of sterile freshly distilled water. This may be injected intravenously, using an appropriate syringe, or preferably it is filtered through cotton into the burette and diluted up to 150 cubic centimetres, using a 0.4 per cent. saline solution or freshly distilled water. In the case of neosalvarsan, since the quantity of solution is only one-half that of salvarsan, it is allowed to enter the vein in five minutes. In all other respects the technic of administration differs in no way from that described for salvarsan.

Intraspinal Administration.—It was soon recognized after the advent of salvarsan that its administration intramuscularly or intravenously in certain forms of syphilitic disease of the nervous system was not so effective as in other manifestations of the disease. This observation was confirmed by the Wassermann reaction, inasmuch as the reaction of the spinal fluid in certain neurological cases persisted positive long after the reaction of the blood-serum became negative. Moreover, it is a common observation to find in syphilitic neurological patients a negative blood and a positive spinal fluid Wassermann or a weakly positive blood reaction and a medium or strongly positive spinal fluid result.

Communication between the lateral ventricles of the brain and the subarachnoid spaces of the brain and spinal cord exists through the foramina of Magendie and Luschka. The nervous choroid plexuses and the velum interpositum, possibly assisted by the blood-vessels of the meninges, act as the chief dividing plane or filtration plant between the blood and the spinal fluid. Owing to the fact that the specific gravity of the blood is greater than that of the cerebrospinal fluid, also that the pressure of the cerebrospinal fluid is greater than that of the intracranial venous blood, osmosis takes place in the direction of the venous blood. This fact may account for the failure of salvarsan,

administered intravenously, to reach the spinal fluid in any considerable quantity. In any event, so far as the effective treatment of cerebrospinal lues, especially paresis and notably tabes dorsalis, is concerned, it prompted a number of investigators to apply both salvarsan and neosalvarsan directly to the subarachnoid spaces by spinal puncture. Wechsellmann,⁴ Marinesco,⁵ Marie and Levaditi⁶ were probably the first to employ this method. The practice was attended frequently with such marked reactions, and even fatalities, that it has already become obsolete. The modifications as suggested by Ravaut⁷ and Wile⁸ using hypertonic solutions of neosalvarsan are likewise not beyond reproach and must be regarded as dangerous procedures, not to be recommended for the average case.

Autosalvarsanized Serum.—Swift and Ellis⁹ have perfected and recommended the intraspinal injection of the patient's own salvarsanized serum, irrespective of the fact that it is impossible by the Marsh test to demonstrate the presence of arsenic in the serum of patients shortly after the intravenous injection of an adult dose of salvarsan, wherefore if present it must be in minute quantity, rendering the *modus operandi* of the apparently beneficial effect of this method of treatment difficult of comprehension. Possibly the antibodies in the serum itself may be a most important factor. It is unquestionably true that autosalvarsanized serum is much less irritating and dangerous than direct subdural injections of salvarsan or even hypertonic solutions of neosalvarsan. Consequently, this method is deservedly

⁴ Deutsch. med. Wochenschr., 1912, 38, 1446.

⁵ Zeitschr. für Phys. und Therap., 1913, 17, 194.

⁶ Bull. et Soc. med. d. hôp., Paris, November 18, 1913.

⁷ Ann. de Med., 1914, 1, 49.

⁸ Jour. Am. Med. Assn., 1914, lxii, 1165; *ibid.*, 1914, lxiii, 137.

⁹ New York Med. Jour., July 13, 1912, 53.



FIG. 66.—Showing position of patient for spinal puncture. The skin has been antisepticized with iodine tincture and the needle introduced through the first intervertebral space above the level of the iliac crests.



FIG. 67.—Lumbar puncture with Strauss needle, illustrating the method of intraspinal injection by the gravity method. After introduction of needle, the obturator, *O*, is withdrawn to point shown in cut. This permits of the spinal fluid to rise in attached pipette, *P*, graduated in hundredths of a cubic centimetre, thereby permitting of the determination of the degree of intraspinal pressure. The desired amount of spinal fluid may then be collected in a test-tube held under the exit, *M*, by removing the metallic plug. The plug is then reinserted, the pressure noted and the obturator partially replaced. The graduated pipette or tube, *P*, is then disconnected from the needle at *C* and the rubber tubing, *T*, leading from the reservoir containing the serum attached to the needle. The obturator is again withdrawn as far as it will go and the serum allowed to flow in. An assistant with his fist in the epigastric region can do much to steady the patient and assist him to properly arch his spine.



FIG. 68.—Intraspinal administration of serum, using syringe. This method has theoretical objections; practically, however, with proper appliances and correct technique, it has proved entirely satisfactory.

the most popular and best form of intraspinal treatment for syphilis thus far proposed.

The technic, slightly modified from the original of Swift and Ellis, is as follows: The patient should receive the same preliminary preparation as for salvarsan or neosalvarsan, of which the former is preferable and more effective. Usually the full adult dose of salvarsan is administered intravenously. Prior to the injection of salvarsan it is assumed that sufficient spinal fluid has been obtained by lumbar puncture (Fig. 66) for the various tests necessary in the control of the number of intraspinal injections, namely, the Wassermann reaction, the lymphocytic count, Noguchi's butyric acid test for globulin or Nonne's phase reaction. One-half to one hour after the intravenous injection of salvarsan, about 40 cubic centimetres of blood are taken in centrifuge tubes from a vein of the opposite arm. This may be allowed to stand until the following day for the separation of the serum or, as is the practice of the authors, immediately centrifuged and 12 cubic centimetres of the serum pipetted off and diluted with 18 cubic centimetres of sterile normal salt solution. This forty per cent. dilution of serum is activated by heating at 56° C. in a water-bath for one-half hour. On the occasions of the second and third treatments, the strength of the serum is commonly increased to 50 and 60 per cent. dilutions. The 30 cubic centimetres of diluted serum are then carefully and slowly injected by lumbar puncture after approximately an equal amount of spinal fluid has been withdrawn. Theoretically, the intraspinal injection is best done by the gravity method (Fig. 67); practically, it can be and is properly accomplished by the faultless use of a syringe, injecting very slowly (Fig. 68). The patient may be permitted to lie on his side or sit with his back arched. Immediately after the injection, the patient must lie down and

the foot of the bed be elevated 14 to 16 inches for four hours. The patient should continue in bed for two or three days. Repetition of the treatment depends upon the extent and duration of any reactions incurred. As a rule the intraspinal injections are repeated at intervals of two weeks. The actual number of treatments should depend upon the analysis of the spinal fluid, the object being to obtain a negative Wassermann reaction, a reduction in the number of lymphocytes to five per cubic millimetre, and a normal globulin content.

Artificially Salvarsanized Serum.—Attempts have been made to salvarsanize serum *in vitro*, in the hope of increasing the arsenical content in view of intraspinal injections. The results have been disappointing and in the employment of neosalvarsan, notably in Los Angeles, disastrous.

Recently Fordyce,¹⁰ adopting the technic of Ogilvie, in which a known amount of salvarsan is added to human serum, recommends the following technic:

“Fifty c.c. of blood are drawn into a centrifuge bottle and centrifuged twice. It is important to have the serum clear and free from fibrin and blood-cells. To obtain the requisite amount of the drug, old salvarsan is mixed in the usual way in the proportion of 0.1 gm. to 40 c.c. of fluid, care being taken not to over-alkalinize; 0.4 c.c. of this solution is the equivalent of 1 mg., and is taken as the standard for measuring the dosage. For this purpose a 1 c.c. pipette graduated in hundredths should be employed. The desired amount of salvarsan is added to from 12 to 15 c.c. of the serum, shaken to and fro to mix thoroughly, and then placed in the incubator at 37 C. (98.6 F.) for one hour, after which it is inactivated for half an hour at 56 C. (132.8 F.). The latter is a most important step in the technic, as Swift and

¹⁰ Jour. Am. Med. Assn., Aug. 15, 1914, 555.

Ellis demonstrated that the spirochæticidal properties of the serum were markedly increased by heating.

“Salvarsanized serum prepared according to the method of Ogilvie must be used fresh, that is, within three hours of the time that it is made up.”

Although a few investigators have added as much as 12 mg. of neosalvarsan to the serum *in vitro* prior to intraspinal injection, with salvarsan an initial dose of 0.1 to 0.25 mg., increased perhaps to 0.5 mg., should not be exceeded. Fordyce regards the limit of safety to lie within 0.5 mg.

After-care of the Patient.—Following a full intravenous injection of salvarsan or neosalvarsan, particularly the former, the patient should lie still for one-half hour. Indeed, it were better for him to be confined to bed over night, and longer if reactions make it desirable. The practice of permitting patients to go home alone immediately after an intravenous injection of the drug is reprehensible, although in many clinics and offices it is commonly done, especially after neosalvarsan. It is our practice both in the hospital and in the office to detain the patient for four to six hours, when if he exhibits no reaction, or any toxic effects of the drug have disappeared, he is allowed to go home. If the reaction is persistent in any way, the patient is advised to remain over night.

After an injection of arseno-benzol, the patient is not allowed to have anything by mouth, even water, for two hours. If, then, he is not nauseated he may be given all the water he cares to drink. Food is usually not craved, but should the patient become hungry during the afternoon or evening a glass of milk, broth or consomme with a slice of bread or a cracker is all-sufficient. As a routine practice, it is unnecessary to treat headache, nausea, vomiting, fever, diarrhœa, etc., which occasionally supervene. Rarely

pyramidon for headache, a mustard plaster to the epigastrium for gastric disturbance, bismuth subnitrate for diarrhoea and an ice-cap for fever may be of some value or at least pacify the patient. Most important is it that the urine be examined the following day, in order to learn the extent of renal irritation, if present. If albumen or casts are found, urinalyses should be performed daily to determine the duration of their persistence, in view of subsequent administration of the drug. If the evidences of kidney irritation are marked or prolonged for more than 24 to 48 hours, the repetition of the injection becomes a serious consideration and as a rule is inadvisable. Arsenical intoxication calls for sweat baths, either electric or hydrothermic. Caution should be enjoined that the patient not exert himself severely in view of cardiac strain for a few days, particularly in the advent of severe reactions.

Clinical Reactionary Effects of Salvarsan.—As a rule clinical reactions are commoner and severer after salvarsan than neosalvarsan. The primary dose, whether the former or the latter, is usually attended with greater reactions than subsequent injections, although the converse of this is frequently observed owing to the cumulative effect of closely repeated administrations. The reactions probably occur by virtue of two facts: Firstly, the endotoxins arising from the destruction of myriad numbers of spirochætae, and, secondly, the toxic effect of arsenic itself, based upon personal idiosyncrasies.

During the administration of salvarsan, the patient not infrequently experiences a sensation of warmth, fulness or throbbing in the head, occasionally complaining of aching of the teeth and a metallic taste in the mouth; his face is usually more or less flushed. If reactions supervene, they are likely to be ushered in by *chilliness* or a definite *chill*

about an hour and a half or two hours after the injection of the drug. This is followed by *headache*, *nausea* and a slight *rise in temperature* to 99–99½ degrees F. *Vomiting* may occur, after which headache and nausea may disappear. In severer types of reaction, nausea and vomiting may persist and recur for several hours, associated with a temperature rise as high as 104 degrees F. *Diarrhœa* is a common occurrence. Rarely a *cutaneous eruption* of an erythematous type due to arsenic may be observed. This is distinctive from the Jarisch-Herxheimer phenomenon, since it may occur in non-syphilitic cases. *Loss of appetite* for a few hours or even a day or two may be observed, and occasionally *irritation of the kidneys*, manifested by a cloud of albumen and a shower of hyaline casts, may occur. This last is a most important warning prohibiting in a few instances the repetition of the intravenous injection of the drug. Careful studies have never revealed injury to a normal heart, kidneys or eyes. It is decidedly reprehensible to administer salvarsan to a patient evidencing renal irritation from mercurial treatment. Weichselmann has ascribed a number of fatalities to this procedure and insists that salvarsan should never immediately follow a vigorous course of treatment by mercury.

In the vast majority of patients, receiving either salvarsan or neosalvarsan, we have observed no sign or symptom other than a trivial transient rise of temperature. Usually all symptoms disappear in six to twelve hours. As a result of treatment, in two or three weeks, the patient invariably feels better physically and mentally; his *anæmia* improves; he gains in weight; all lesions rapidly disappear and the patient is inclined to construe the symptomatic into a permanent cure.

The Jarisch-Herxheimer Reaction.—This phenomenon,

originally associated only with a characteristic cutaneous eruption following antisyphilitic treatment, at one time—before the discovery of the *treponema pallidum* and the Wassermann reaction—enjoyed considerable importance as a diagnostic aid. The skin and mucosa reactions are manifested by œdema, swelling, redness, pain and other inflammatory signs. To-day the Herxheimer reaction is regarded to be much broader in its scope and may be defined as *a cutaneous eruption, an aggravation of a pre-existent syphilitic roseola or any inflammatory reaction in syphilitic tissue provoked by the administration of salvarsan, neosalvarsan or mercury*. This is to be distinguished from the arsenic rash in non-syphilitics receiving salvarsan. Various manifestations of the reaction are apparent. The increased redness of the roseola, the inflammatory reaction in mucous patches, the swelling, exudation and occasional ulceration of gummata observed a few hours or a day or so after medication, the lancinating pains of *tabes dorsalis*, augmented or relighted, as observed following the Swift-Ellis treatment, the so-called neuro-recurrences and the provocative positive Wassermann reaction are all evidences of the Herxheimer reaction in whole or in part.

According to Ehrlich the occurrence of these phenomena is due to treatment dosage insufficient to destroy completely the *treponemata pallida* whereby the escaped viable organisms are sensitized or stimulated to increased activity.

Excretion of Salvarsan from the Body.—The elimination of salvarsan from the system has been carefully studied both on the human and on lower animals. It has been determined by urinalysis and gastric analysis that the elimination of the drug occurs almost immediately following its intravenous administration, while after intramuscular injection it is seldom detected for an hour, although after subcutaneous in-

jection it has been observed as early as twenty-five minutes. Fischer and Hoppe claim that after intravenous injections no arsenic is demonstrable after two to three days, while after subcutaneous injections the time limit is four to five days; after intramuscular injections the urine may show the presence of arsenic for six to ten days. Consequently it is the natural supposition and the case that the duration of elimination following intramuscular and subcutaneous administration is longer than after the intravenous injection. It is alleged that, after the last method of therapy, arsenic has been found in the blood at the end of two days, and absent at fourteen days; in the urine for two or three days; in the stools for five or six days. By the Marsh test the authors have been unable repeatedly to demonstrate the presence of arsenic in the blood-serum thirty, forty-five and sixty minutes after the intravenous injection of salvarsan or neosalvarsan, nor have they succeeded in demonstrating traces of the drug in the spinal fluid one and a half to two hours following intravenous administration. It is possible that the mould test of Gosio, the most delicate qualitative test for arsenic that we have, may throw new light on this subject. The test depends upon the fact that a certain mould (*Penicillium brevicaulis*) when cultured on a medium containing arsenic produces a distinct odor of garlic. Examination of human milk at the end of three and twenty-four hours demonstrated the absence of the drug in that secretion. From animal experiments it is proper to infer that the drug is entirely eliminated from the liver and bone marrow in ten to sixteen days. Further experiments show that mercury delays the excretion of arsenic and explains why and how mercury ably supplements and supports salvarsan and neosalvarsan in the treatment of syphilis. Potassium iodide, on the other hand, accelerates the elimination of arsenic.

Results of Specific Treatment in Syphilis.—We have four ways of measuring the effect of salvarsan in the treatment of syphilis: (1) the disappearance of *treponemata pallida*, (2) the disappearance of lesions, (3) the recurrence of lesions and (4) the Wassermann reaction. Of these, the first, constituting the clinical symptomatology, is the most important. However, the value of the Wassermann reaction, correctly and competently performed, in the diagnosis and control of the treatment of syphilis, must not be underestimated, since, by its use, the diagnosis in doubtful cases may be determined and by its periodic utilization, recurrence of lesions may be avoided.

The earlier and more intensive the antisyphilitic treatment, whether by salvarsan or mercury, the sooner and more lasting are negative results to be expected. A single negative result serologically does not signify cure and is commonly observed in the treatment of early syphilis; the Wassermann reaction may rapidly return to positive if the amount of treatment has been insufficient. On the other hand, the duration of negative reactions is directly proportional to the thoroughness of treatment. McIntosh and Fildes state that positive reactions in the secondary stage of syphilis may be expected to become negative after a single course of mercury in 66 per cent. of cases; in tertiary syphilis in 33 per cent. of cases. One-half to two-thirds of patients have negative reactions after ten courses of mercury or two years of pills. It is safe to conclude that one-third to one-half of all patients treated by the old-fashioned method, although exhibiting no signs or symptoms, judged from the serological standpoint are or were not cured of syphilis. May this large percentage not include many of those patients who later in life develop *tabes dorsalis* and *paresis*?

What has salvarsan added to the curability of syphilis?

McIntosh and Fildes state after the intramuscular and subcutaneous administration of salvarsan (0.8 to 1.2 gm.) in primary cases all showed negative Wassermann reactions on an average of five weeks; in the secondary stage many became negative on an average of eight weeks; in the early latent form the average was ten weeks; in the tertiary stage little or no effect was observed so far as negative reactions were concerned. After the combination of intramuscular with intravenous injections (0.8 to 1.7 gm.), however, a much more pronounced beneficial effect was observed. In secondary syphilis no case failed to become negative and did so with great regularity in six and a half weeks. In the tertiary stage of the disease the intravenous method again proved its superiority over the intramuscular and was productive of a high percentage of negative reactions. The dictum of Ehrlich, that in the use of salvarsan there exists an agent capable of curing syphilis by a single dose—a “*therapia sterilisans magna*”—is true. Our experience has shown that if the diagnosis of the chancre be made sufficiently early, using the dark field ultra-microscope, before the Wassermann reaction becomes positive, the disease may be cured by a single dose of salvarsan. The hope inspired by that dictum, however, has not been fully realized, in view of the fact that only a small percentage of cases, untreated with mercury, yield a permanently negative Wassermann reaction even after repeated injections of salvarsan. It is the consensus of opinion to-day that, as a general rule, the most appropriate, efficient and speedy cure of syphilis is the association of salvarsan, preferably intravenously, with mercury or mixed treatment.

GLOSSARY

- Active Immunization.***—The process by which the body cells of an animal are stimulated by a toxin or foreign body (antigen) to the production of antibodies specific against the given foreign substance.
- Agglutination.***—A phenomenon characterized by clumping and loss of motility of bacteria, brought about by agglutinins.
- Agglutinins.***—Antibodies of the second order of Ehrlich, producing agglutination.
- Allergy.***—The altered condition of an animal into whose tissues has been introduced an antigen or foreign cell product.
- Amboceptor.***—Specific antibody of the third order of Ehrlich, which acts only in conjunction with non-specific substance or complement.
- Anaphylaxis.***—A series of apparently deleterious effects produced by a second injection of specific protein material into an animal that has been previously "sensitized" by a prior injection of the same material.
- Antigen.***—A foreign substance, usually of protein nature, capable of exciting the formation of specific antibodies.
- Antiserum.***—Serum containing specific substances whereby the action of bacteria or their toxins is antagonized.
- Antitoxin.***—Specific substance produced in the blood-serum whereby the action of bacterial toxins is antagonized.
- Autogenous Bacterin.***—Therapeutic suspension of bacteria prepared from the particular strain cultured from the infected patient.
- Autolysis.***—Disintegration of bacteria by treating them with salt solution, alcohol, ether, chloroform, etc., theoretically to remove toxic or antiopsonic substances.

Bacterin.—A suspension of bacteria prepared for therapeutic purposes.

Bacteriocidin.—Substance in the blood-serum, capable of destroying bacteria.

Bacteriolysin.—Specific antibody of third order of Ehrlich, concerned in the dissolution of bacteria.

Bordet-Gengou Phenomenon.—Complement-fixation reaction as first applied to infection with cholera spirilla.

Complement.—Non-specific substance normally present in all blood-serum, acting in conjunction with antibodies of third order of Ehrlich.

Complement-fixation.—The using up, and rendering unavailable for further use, of complement, in the reaction between antigens and their specific antibodies (amboceptors).

Cytolysin.—Specific antibody of third order, capable of dissolving foreign cells.

Endotoxin.—Toxin bound up in the bacterial protoplasm, and only set free by disintegration of the bacteria.

Hæmolysin.—Specific antibody of the third order of Ehrlich, capable of disintegrating foreign red blood-cells.

Hæmolysis.—Disintegration of red blood-cells, setting free the hæmoglobin.

Hæmolytic Amboceptor.—Synonymous with hæmolysin.

Immunity.—The resistance manifested by man and various animal species to infectious microorganisms or other foreign proteins.

Immunization.—The process by which the state of immunity is attained. It comprises two forms, *active* and *passive*.

Lysins.—Specific antibodies of third order of Ehrlich, comprising cytolysins, hæmolysins, bacteriolysins, etc.

Opsonic Index.—Measure of the ratio of the phagocytic activity of neutral washed leucocytes in the patient's

serum for given bacteria, as compared with those in a normal or control serum.

Opsouins.—Specific substances in the blood-serum possessed of the ability to sensitize or prepare bacteria for phagocytosis.

Organotherapy.—Therapeutic administration of products of ductless glands and other organs.

Passive Immunization.—The process by which immunity is acquired when artificial antisera are injected into the animal body.

Phagocytosis.—Property of the leucocytes whereby they take up into their substance foreign particles, such as bacteria, pigment, carbon granules, etc., thus removing them from the circulation.

Precipitins.—Antibodies of the second order of Ehrlich, formed in the blood in response to unorganized protein material.

Sero-bacterin.—Bacterins prepared by treating bacteria with their specific immune serum, thus “sensitizing” them so that they are acted upon by the complement in the patient’s blood immediately after injection.

Stock Bacterin.—A therapeutic suspension of bacteria which have been isolated from another patient, who has suffered from a similar infection.

Toxins.—The soluble products of bacterial and plant growth, whereby their deleterious effects are brought about.

Tuberculin.—Various preparations from tubercle bacilli for therapeutic and diagnostic purposes.

Vaccination.—Protective immunization to smallpox by inoculation with cow-pox virus.

Vaccine.—Term properly reserved for cow-pox virus; now frequently applied to therapeutic suspensions of bacteria or bacterins.

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